*Targeting**Impulsivity***

Filip Sebastiaan van den Bergh

ISBN-10: 90-393-4247-4
ISBN-13: 978-90-393-4247-3

Printed by Ponsen & Looijen b.v., Wageningen
Cover design by Conny Groenendijk/Filip van den Bergh
Cover photo by Henk Bok

*Targeting**Impulsivity***

Focus op Impulsiviteit

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. W. H. Gispen,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op donderdag 1 juni 2006
des middags te 2.30 uur

door

Filip Sebastiaan van den Bergh

geboren op 2 mei 1979 te Dordrecht, Nederland

Promotores: Prof. dr. B. Olivier
Prof. dr. J. L. Kenemans

Copromotores: Dr. R. S. Oosting
Dr. L. Groenink

Dit proefschrift werd (mede) mogelijk gemaakt dankzij financiële steun van:
Psychogenics, Inc.
Jurriaanse stichting

Response inhibition deficits and delay aversion: Differences and similarities

Just assume for a moment that it's not hypothetical.
Hans Teeuwen

Introduction

Uncertain situations or rapidly changing environments demand rapid responding, and humans and animals are well equipped to do so. When swift actions are called for, humans and animals can adopt a heuristic decision-making process, acting fast and using limited information. However, when an individual consistently exhibits rash, reckless or thoughtless behavior even in inappropriate situations, that individual is considered impulsive. Individuals suffering from severe impulsivity are severely hindered in their normal functioning: they have difficulties in school, and are unable to hold a job or maintain a relationship. As such, impulsivity is a major symptom of many mental disorders listed in DSM-IV, such as attention-deficit hyperactivity disorder, mania, intermittent explosive disorder, and pathological gambling (2000). As a theoretical construct, impulsivity is rather ill defined. Although impulsivity is no longer seen as a single concept by preclinical researchers, this notion has not entered the clinics yet (Evenden 1999).

The aim of this thesis is to find new drug targets for the treatment of impulsivity. To that end, we review and extend an existing division of impulsivity into two different subtypes: deficits of response inhibition and delay aversion. In the first case, impulsive individuals have difficulty inhibiting planned or ongoing behavior if the behavior is no longer appropriate. Also, impulsive individuals have difficulty shielding planned or ongoing behavior from disruption by irrelevant stimuli (Barkley 1999; Quay 1997). The second impulsivity subtype is the result of an inability to wait for gratification. The value of rewards decreases with increasing delays to the availability of that reward. For impulsive individuals, this decrease in value is faster than for non-impulsive individuals (Ainslie 1975; Sagvolden et al. 1998; Sonuga-Barke et al. 1992). Response inhibition and delay aversion have recently been unified into a single theory as separate but complementary processes that can both lead to impulsive behavior (Johansen et al. 2002; Kuntsi et al. 2001; Nigg et al. 2004; Solanto et al. 2001; Sonuga-Barke 2005). Although both response inhibition deficits and delay aversion can lead to similar impulsive symptoms, the underlying neural substrate and thus treatment may be very different.

In the first part of this chapter, response inhibition deficits and delay aversion will be reviewed as causes of impulsivity. The focus will be on the significance of the subtypes of impulsivity on everyday life, the methods available for measuring impulsivity subtypes in the laboratory, and the pharmacology and neural substrate underlying the subtypes. The aim of the first part is to establish the impulsivity subtypes as separate entities. In the second part of this chapter, the similarities and relationships between the two subtypes will be discussed. The aim of the second part is to suggest a framework for the integration of the two impulsivity subtypes. Finally, this introduction will be closed by an overview of this thesis.

Response inhibition deficits

Response inhibition is the ability to inhibit planned or ongoing motor responses when they are no longer appropriate, or to protect ongoing or planned behaviors from interference (Barkley 1999). A lack of response inhibition is not necessarily pathological: some situations require more self-control than others. A dangerous situation calls for rapid changes of behavior and openness to outside stimuli, while a mentally challenging effort requires determination in ongoing and planned behaviors. A non-impulsive individual is able to adjust behavioral inhibition according to the situation, while an impulsive individual displays a more consistent failure to inhibit behavior or ignore interference. To illustrate inhibitory control, imagine a cup falling off a table. The correct reflex would be to attempt to catch the falling cup, and preventing it from breaking. While many of us will display this reflex, it is not always appropriate. If the falling object would have been a sharp knife, it is better to let it drop to the floor than to try and catch it with bare hands.

Inhibitory control is usually visualized as a horse race: a competition between hypothetical excitatory and inhibitory signals (Logan 1994). Applying this model to our example, the knife is recognized, the plan to catch the knife is formed, the response is prepared, and then executed. When another part of the brain decides against catching the knife, it initiates an independent inhibitory signal. If the inhibitory signal is able to catch up with and disrupt the excitatory signal before it reaches a point-of-no-return, it wins and the response is inhibited. The knife is thus allowed to fall.

Measuring response inhibition

The stop-signal task is the most often used measure of response inhibition in humans. In the basic form of the stop-signal task, subjects respond to stimuli presented on a computer screen by pressing a key on a keyboard (the go-stimuli). For example, subjects press a button each time a '>' is presented on the screen, and another button if an '<' is presented. However, in a percentage of randomly selected trials, usually around 25%, in addition to the go-stimulus a second stimulus is presented (the stop stimulus) signifying that the subject should withhold all responses. The stop stimulus (usually an audible signal) is presented after a certain interval following the go-stimulus, and occurrence of the stop-stimulus is completely unpredictable to the subject. By varying the interval between the go-stimulus and the stop-stimulus, the researcher has some control over the success the subject will have in inhibition. Inhibition success rates and an estimation of the reaction time of the inhibitory process are both used as indices of response inhibition (Logan 1994). The stop process reaction time has to be estimated because, if an inhibition was successful, there is no response, and therefore no reaction times are measured (Appendix A). In 2003, this task has been adapted for use in rats (Eagle and Robbins 2003a; b). In the rat version, rats are trained to rapidly respond on two levers in succession. On trials in which the stop-signal is presented in the interval between the two lever responses, rats are to withhold responding on the second lever. The same basic principles that have been used for

human subjects can be applied to rats as well.

The stop-signal task is not the only task measuring response inhibition. In the stop-change task, subjects respond to the stop signal by executing an alternative response. Whether these two tasks measure the same type of response inhibition is still under debate (De Jong et al. 1995; van Boxtel et al. 2001).

The five-choice serial reaction time task is an animal analog of the human continuous performance task (Carli et al. 1983). In the five-choice serial reaction time task, animals are trained to respond to brief (usually under 1 s) stimuli presented randomly in one of five apertures. The success of the animals in making a response in the correct hole is a measure of attention. Since the animal is primed to respond in one of the holes, these responses are sometimes executed even before the stimulus is presented. These anticipatory responses may be a measure of impulsivity (Robbins 2002). No research, however, has directly investigated the type of impulsivity measured in the five-choice serial reaction time task, and it is therefore unclear whether the five-choice serial reaction time task is a measure of the same type of inhibitory control measured in the stop-signal task.

Because response inhibition is a fundamental process, it is a component of many other measurements besides those summed up above. Extinction learning is also often understood in terms of competing excitatory and inhibitory signals. In extinction a previously reinforced response is no longer rewarded. As a result, the frequency of the response decreases. Fast re-learning and spontaneous recovery after having left the environment for a period of time indicate that the stimulus-response association is not forgotten, but the subject has learned that the stimulus-response contingency has changed (Rescorla 2004). Extinction is impaired in patients suffering from ADHD and in the SHR, an animal model for ADHD (Johansen et al. 2002; Johansen and Sagvolden 2004; Sagvolden et al. 1998; Sagvolden et al. 1992). Finally, aggression or irritability may be mediated by poor response inhibition as well (Brunner and Hen 1997). The aggressive act is executed without forethought, as signified by descriptions such as "hair-trigger" responses (Barratt et al. 1997). Impulsive and aggressive behaviors go together in many mental disorders, such as ADHD, Conduct disorder, and Oppositional Defiant Disorder (Turgay 2005).

The psychopathology of response inhibition deficits

The stop-signal reaction time task in humans is one of the most applied tests for measuring impulsivity, and many studies are available in patients suffering from a wide variety of different mental disorders. Both children (Konrad et al. 2000; Schachar et al. 2000) and adults (Aron et al. 2003; Bekker et al. 2005; Murphy 2002; Wodushek and Neumann 2003) suffering from attention-deficit hyperactivity disorder show decreased stop reaction times. Aggressiveness can be the result of dysfunctional inhibitory control as well (Oosterlaan and Sergeant 1996). Patients suffering from other mental disorders, such as autism (Ozonoff and Strayer 1997) or schizophrenia (Badcock et al. 2002) do not display altered performance. Patients suffering

from obsessive-compulsive disorder, on the other hand, show faster stop-signal response times (Krikorian et al. 2004). In addition, several studies report on the effects of physical trauma to various parts of the brain on stop-signal task performance (Dimitrov et al. 2003; Rieger and Gauggel 2002; Rieger et al. 2003; Stewart and Tannock 1999), including Parkinson's (Gauggel et al. 2004) and Alzheimer's disease (Amieva et al. 2004).

Conforming to the notion that stop-signal task performance and extinction speed are reflections of the same underlying process, children suffering from attention-deficit hyperactivity disorder are reported to respond twice as much as controls during extinction sessions (Sagvolden et al. 1998).

Pharmacology of response inhibition

Relatively little is known about the pharmacology of inhibitory control, because until the recent development of the stop task in animals, there was no clear measure of inhibitory control in animals. Most pharmacological studies have therefore been done in humans, or in animal tasks in which the nature of the impulsivity measured is less clear, such as the five-choice serial reaction time task.

Dopamine

Although the main mechanism of action of psychostimulants such as D-amphetamine or methylphenidate is blocking the dopamine transporter and releasing dopamine, thereby causing elevated dopamine concentrations in the synaptic cleft, many psychostimulants bind to other proteins as well, including the serotonin and norepinephrine transporter (Gatley et al. 1996). Methylphenidate elevates extracellular dopamine and norepinephrine levels, and D-amphetamine elevates synaptic serotonin levels in the prefrontal cortex of rats, in addition to the effects mentioned for methylphenidate (Kuczenski and Segal 1997). In humans, methylphenidate and D-amphetamine have been proven effective in lowering impulsivity in the stop-signal task (Aron et al. 2003; de Wit et al. 2002; Potter and Newhouse 2004). L-dopa, a dopamine precursor, has no effect on stop-signal task performance, suggesting that the efficacy of methylphenidate is not solely attributable to its effects on the dopamine transporter (Overtoom et al. 2003).

Impulsivity-decreasing effects of psychostimulants in the five-choice serial reaction time task are found only under specific conditions. When impulsive responses are registered but not punished, psychostimulants decrease impulsivity slightly (Bizarro et al. 2004). In addition, dopamine depletion in the dorsal striatum does not alter the amount of impulsive responses in the five-choice serial reaction time task (Baunez and Robbins 1999). This suggests that the type of impulsivity measured in the five-choice serial reaction time task is not dopamine dependent. As stated before, the type of impulsivity measured in the five-choice serial reaction time task is unclear.

Serotonin

Naturally occurring polymorphisms of the serotonin transporter gene are found to be associated with impulsivity (Lee et al. 2003) and violence (Retz et al. 2004), indicating a potential involvement of serotonin in response inhibition. Local serotonin depletion leads to a failure of response inhibition in several species and a number of tests. For example, lowering serotonin by L-tryptophan (a serotonin precursor) depletion in healthy human volunteers results in an increase in impulsive responding as measured by a stop-signal task (Crean et al. 2002) and a continuous performance task (Walderhaug et al. 2002). Depletion by intracerebroventricular administration of 5,7-DHT of rats leads to impulsivity as measured in the stop-signal task (Harrison et al. 1999) and the five-choice serial reaction time task (Harrison et al. 1997; Winstanley et al. 2004c). Serotonin depletion by systemic injection of PCPA in rats leads to an increased responding during extinction trials (Beninger and Phillips 1979), providing further evidence of possible a link between response inhibition and extinction.

But there is much more to the serotonin system than serotonin transporter polymorphisms and serotonin depletions. The serotonin system consists of fourteen receptors (Barnes and Sharp 1999), and not all of them are involved in impulsivity. Stimulation of the receptors that are involved may even result in opposite effects. For example, in the five-choice serial reaction time task, stimulating the 5-HT_{2A} receptor generally decreases impulsivity (Winstanley et al. 2003a; Winstanley et al. 2004c), while stimulation of the 5-HT_{2C}-receptor generally increases impulsivity (Winstanley et al. 2004c).

Finally, aggression may be the result of dysfunctional response inhibition (Barratt et al. 1997; Brunner and Hen 1997; Oosterlaan and Sergeant 1996), and pathological aggression may be treated with a class of drugs called serenics, agonists of serotonin 1A and 1B receptors (Olivier et al. 1994; Olivier and van Oorschot 2005). The effects of serenics have been shown to be the result of their activity at postsynaptic 5-HT_{1B}-receptors (Olivier and van Oorschot 2005), and mice lacking 5-HT_{1B}-receptors display increased aggression and decreased inhibition (Bouwknecht et al. 2001; Brunner and Hen 1997). Serenics thus have a putative anti-impulsive effect.

Norepinephrine

The role of norepinephrine in response inhibition is not very clear. Although atomoxetine, a norepinephrine reuptake inhibitor, is effective in patients suffering from attention-deficit hyperactivity disorder (Corman et al. 2004; Pataki et al. 2004), relatively few reports have addressed the effects of noradrenergic drugs in one of the tasks for response inhibition discussed above. Depletion of forebrain norepinephrine slows extinction of several learned responses, indicating a possible role for norepinephrine in response inhibition (Mason and Iversen 1977). Furthermore, desipramine (a norepinephrine reuptake inhibitor) has been demonstrated not only to be effective in attention-deficit hyperactivity disorder (Greydanus et al. 2002; Maid-

ment 2003), but also to increase stopping performance in a human stop-signal task (Overtoom et al. 2003). Methylphenidate is known to act at the norepinephrine transporter in addition to its dopaminergic properties, perhaps adding to the therapeutic effects of the drug (Gatley et al. 1996). It has been suggested that norepinephrine levels are especially important in their relationship to dopamine levels: children suffering from attention-deficit hyperactivity disorder have a lower norepinephrine activity as compared to dopamine activity than do controls (Oades 2002; Pliszka et al. 1996). These theories mainly address attention and attention-deficit hyperactivity disorder, suggesting norepinephrine is especially important in attention.

Neural substrate of inhibitory control

Imaging and EEG measures strongly indicate the frontal cortex as the brain area mediating response inhibition (Casey et al. 1997). During stop-signal task measurements, the frontal cortex seems to be especially important for stopping behavior (Band and van Boxtel 1999; Overtoom et al. 2002; van Boxtel et al. 2001). In addition to psychophysiological data, both accidental lesions in patients and intentional lesions in animals signify the importance of the frontal cortex in response inhibition. Patients with lesions of the frontal cortex (in particular the right half) show significantly longer stopping speeds than controls (Rieger et al. 2003). Rats with lesions of the prefrontal cortex show an elevated tendency to respond impulsively in the 3-choice serial reaction time task, a variant of the task described above (Broersen and Uylings 1999). For an of overview of the data supporting the frontal cortex as a locus of inhibition upstream to the motor cortex, see Band and Van Boxtel (1999). Unexpectedly, rats with medial prefrontal lesions do not perform worse in the stop-signal task (Eagle and Robbins 2003b). Eagle and Robbins suggest this lack of effect may be due to the other area of importance for stopping behavior: the basal ganglia. The basal ganglia are thought to complement the frontal cortex in its inhibitory function, although the exact mechanism is still unclear (Band and van Boxtel 1999). Like patients with damage to the frontal cortex, patients that have suffered damage to the basal ganglia show impaired performance on a stop task (Rieger et al. 2003).

Delay aversion

Impulsivity can also be described as an aversion to waiting for rewards, often called delay aversion, faster temporal discounting, or impulsive choice (Ainslie 1975; Barkley et al. 2001; Sagvolden et al. 1998). Impulsive individuals that display delay aversion will choose an option that does not require waiting or try to decrease the subjective waiting time by taking attention away from the reward and the delay to obtain it. Like a failure of inhibitory control, delay aversion is not necessarily pathological. Some situations require taking a more conservative approach, while others may require squandering. Imagine you have obtained a large amount of money. Spending everything the next day without saving some for more difficult times is impulsive, but keeping money in the bank without ever using it may also be inappropriate. Animals that hoard food face a similar conflict. If an individual displays a consistent delay aversion

without being able to adjust to the situation, the individual is characterized as impulsive.

The underlying principle in delayed reward paradigms is the discounting principle: the value of a reward diminishes with the delay to obtaining the reward. The speed by which the reward loses value is not a constant; rather it is described by a hyperbola: fast at first and more slowly for longer delays (Green et al. 2005; Ho et al. 1999; Holt et al. 2003). In addition, the speed by which rewards lose their value is dependent on the amount of the reward (Johnson and Bickel 2002). For additional information on the rat delayed reward task and hyperbolic discounting, see Appendix B.

Measuring delay aversion

In a delayed reward procedure, subjects make a choice between a small, immediately available reward and a large but delayed reward. Delay discounting has been observed in many species, including humans (Kirby et al. 1999; Reynolds et al. 2004), rats (Bizot et al. 1999; Evenden and Ryan 1996), mice (Isles et al. 2003), and pigeons (Wolff and Leander 2002). Humans generally fill out paper or computer-aided questionnaires, while animals make lever or nosepoke responses (Alessi and Petry 2003; Evenden and Ryan 1996; Holt et al. 2003; Reynolds et al. 2004; Thiebot et al. 1985). The obtained rewards are also different: humans are generally rewarded with money (sometimes the rewards are hypothetical), and animals with food or water. Despite these differences, both methods yield very similar measures of delay aversion.

Originally, the delayed reward task in rats was conducted in a three-arm maze (Bizot et al. 1988; Thiebot et al. 1985), but recent versions are operant tasks. Animals choose between two levers, one delivering a small but immediate reward, the other a large but delayed reward. In the adjusting version of the operant task, the choices the animal makes determines the length of the delay: a choice for the delayed reward increases the delay to the next trial, while a choice for the immediate reward decreases the delay (Ho et al. 1999; Mobini et al. 2000a). Problems with this approach make interpretation of the results difficult (Cardinal et al. 2002), and recent research often uses a different approach in which the delays are not adjusted as a result of animal's choices (Cardinal et al. 2000; Evenden and Ryan 1996; 1999; van Gaalen et al. 2005). In the adjusting version of the task, animals switch between the response alternatives throughout the session to maintain a steady state, while in the non-adjusting version of the task, only one switch is necessary (although more may be made by the animal). Such task differences make comparisons between results obtained with the two tasks difficult.

Discounting procedures can be used with other manipulations that decrease the value of a reward as well. For example, in a probabilistic reward task, subjects choose between reinforcers differing in size and probability (Ho et al. 1999; Rachlin et al. 1991; Reynolds et al. 2004). A small reward can be obtained with certainty, while the larger reward can be obtained with a probability less than 1 (Ho et al. 1999; Rachlin et al. 1991; Reynolds et al. 2004). This task seems to represent a mechanism that is different from the mechanism underlying the delayed

reward paradigm (Green and Myerson 2004; Green et al. 1999; Mobini et al. 2002; Mobini et al. 2000b). Effort discounting is another type of discounting, in which the larger reward is obtained only with an investment of more effort (Mitchell 2004; Silva and Gross 2004). These alternative discounting procedures may prove very valuable in dissociating time perception from impulsivity in delayed reward tasks.

The psychopathology of delay aversion

Children suffering from attention deficit hyperactivity disorder and oppositional defiant disorder display a greater temporal discounting of rewards than controls (Barkley et al. 2001; Solanto et al. 2001). Faster discounting is also observed in alcoholics (Mitchell et al. 2005), heroin-abusers (Kirby et al. 1999), cocaine-abusers (Coffey et al. 2003), and smokers (Reynolds et al. 2004). Delay aversion is not just associated with substance abuse, as gambling addicts also display delay aversion (Alessi and Petry 2003), suggesting that the used substances do not induce delay aversion. Rather, delay aversion predisposes individuals to addiction. Impulsivity as measured in a delayed reward paradigm predicts acquisition of intravenous cocaine self-administration in rats (Perry et al. 2004), further suggesting a delay aversion as a trait predisposing for addiction. Opiates themselves, however, may still lead to impulsivity in the delayed reward task (Kieres et al. 2004).

Pharmacology of delay aversion

Animal delayed reward procedures have been available since 1985 (Thiebot et al. 1985), and the effects of many psychoactive substances have been studied. Unfortunately, as stated above, the use of different procedures in literature makes interpretation difficult.

Dopamine

Several studies have confirmed the beneficial effects of psychostimulants in delayed reward tests. Psychostimulants lower impulsivity both in humans (de Wit et al. 2002; Pietras et al. 2003) and in rats (Cardinal et al. 2000; van Gaalen et al. 2005; Wade et al. 2000). As stated above, the exact method can have a significant impact on outcomes of drug tests. The effects of D-amphetamine illustrate this difficulty. If the delay to the large reward is signaled, effects of D-amphetamine are more robust then when the delay is not signaled (Cardinal et al. 2000). In a recent study, however, D-amphetamine lowers delay aversion even without signaling the delay (van Gaalen et al. 2005). Other dopamine agonists, such as methylphenidate and GBR12909 have the same effect in rats (van Gaalen et al. 2005). Selective dopamine receptor agonists and antagonists provide some insight into the dopamine subsystem involved in the alleviating effects of psychostimulants. The dopamine D_1-receptor antagonist SCH23390 increases delay aversion, but eticlopride (a dopamine D_2-receptor antagonist) has no effects (van Gaalen et al. 2005). When co-administered with D-amphetamine, however, eticlopride blocked its anti-impulsive effects, indicating an involvement of the dopamine D_2-receptor in the effects of D-amphetamine.

Serotonin

The effects of manipulations of the serotonergic system on delay aversion are complex and inconsistent. First, the different delayed reward tasks interact with serotonergic manipulations. In a non-adjusting procedure in rats, serotonin depletion by local infusion of the neurotoxin 5,7-DHT has no effects (Winstanley et al. 2003b; 2004a), but in adjusting procedures, impulsivity increases (Mobini et al. 2000a). In pigeons, treatment with selective serotonin reuptake inhibitors decreased delay aversion in a adjusting task (Wolff and Leander 2002). These discrepancies may be the result of a fundamental difference between adjusting and non-adjusting delayed reward tasks. In non-adjusting delayed reward tasks, animals choose the large reward until the subjective value of the large reward is decreased by the delay to a value smaller than that of the small reward. Thus, such procedures require at least only one switch. In adjusting procedures, animals switch between the response alternatives throughout the session, thereby maintaining the delay at a steady level. Non-adjusting delayed reward tasks therefore require less behavioral flexibility than adjusting delayed reward tasks. From reversal procedures it is known that serotonin plays an important role in cognitive flexibility. In a reversal procedure, a previously reinforced response no longer delivers any rewards, while another previously non-reinforced response is being reinforced. Serotonin depletion in the prefrontal cortex of marmoset monkeys leads to cognitive inflexibility (Clarke et al. 2004). Similar results are found in tryptophan-depleted human subjects, in which depletion of the serotonin precursor tryptophan leads to lowered serotonin activity (Rubinsztein et al. 2001). Decreasing cognitive flexibility in the delayed reward task has also been reported by Evenden and Ryan (1999), who show that animals administered a low dose of 8-OH-DPAT (a 5-HT_{1A}-receptor agonist) make responses that appear to be more random and less dictated by the delay. At very high doses of 8-OH-DPAT (1 mg/kg), animals choose the small reward almost exclusively (Winstanley et al. 2005).

Second, assuming that execution of any operant task requires more cognitive flexibility early in training, manipulations of the serotonin system may also differ importantly between novice and well-trained animals. This may account for discrepancies between studies. For example, 8-OH-DPAT sometimes affects delay aversion (Evenden and Ryan 1999), and is sometimes ineffective at the same dose (Winstanley et al. 2005).

Third, stimulation of the different serotonin receptors may cause opposite effects in the delayed reward task (Evenden and Ryan 1999). As described above, 5-HT_{1A}-receptors are involved in delay aversion, even though its role is still unclear. DOI, a 5-HT_2-receptor agonist increased delay aversion. Antagonists of 5-HT_2 and 5-HT_3-receptors have no effect on delay aversion, suggesting that the circuitry involved in delay aversion is not under tonic control of serotonin.

And finally, after chronic administration, important changes occur in the serotonin system, which possibly account for the delay in the therapeutic effects of serotonergic drugs (Belzung et al. 2001). In the delayed reward task, buspirone (a partial 5-HT_{1A}-receptor agonist) acutely induces impulsive choice, while the same drug lowers delay aversion after chronic administra-

tion. Because WAY-100635, a 5-HT_{1A}-receptor antagonist blocks the effects of buspirone on impulsive choice, effects on receptors other than 5-HT_{1A}-receptors cannot account for this finding (Liu et al. 2004).

This leaves us to conclude that, while serotonin is involved in the execution of the delayed reward task, its exact role in delay aversion is still largely unclear.

Norepinephrine

The role of norepinephrine in delay aversion is not very well studied. Van Gaalen and colleagues (van Gaalen et al. 2005) found some effects of desipramine in the delayed reward task. Unfortunately, their uncorrected post-hoc tests led to an overestimation of the significance of the results of desipramine administration.

Neural substrate of delay aversion

Subjective reinforcer values are thought to result from dopaminergic signaling in reward areas of the brain, including the ventral tegmental area and the nucleus accumbens (Sonuga-Barke 2003). Dopaminergic neurons from the ventral tegmental area project to the nucleus accumbens, the striatum, and the prefrontal cortex (Kandel et al. 2000). The mechanism by which dopamine signals subjective reward value is still unclear (Salamone and Correa 2002).

The prime area involved in waiting for delayed rewards is the nucleus accumbens core. Rats with lesions to this area, but not the frontal cortex, display an impulsive profile in a delayed reward task (Cardinal et al. 2001). This finding is in accordance with the pharmacology of the delayed reward task, as many dopaminergic neurons are found in that area (Glaser et al. 2005). Two other structures involved in execution of the delayed reward task are the orbitofrontal cortex and the basolateral amygdala (Winstanley et al. 2004b). Animals with lesions to the orbitofrontal cortex are unable to update the changing value of rewards as the delay increases (Winstanley et al. 2004b). In a competing view, Kheramin and colleagues (2002) relate the altered performance of orbitofrontal-lesioned rats in the delayed reward task to a decrease in immediate reward values combined with a slower discounting rate. Their interpretation may be confounded by their use of a hyperbolic discounting function, because the applicability of this function in lesioned animals is unclear. Lesions of the basolateral amygdala induce impulsive choice in rats, and it is suggested that animals are unable to maintain a representation of the reward value during delays (Winstanley et al. 2004b). The basolateral amygdala has been shown to be important for maintaining reward representations through dopaminergic mechanisms in the striatum in other tests as well (Cador et al. 1989).

Integrating impulsivities: the serial process model

The previous paragraphs have demonstrated a clear difference on several levels between response inhibition deficits and delay aversion as possible causes for impulsivity. This distinction, however, has not made its way into clinical practice yet. Patients with ADHD are a very heterogeneous group, some suffering from response inhibition deficits (15%), some from delay aversion (27%), while others display both impulsivity subtypes (29%) (Sonuga-Barke 2003). All of these patients are treated similarly. Recognition of the heterogeneity of impulsivity may lead to better and more specific treatments.

In hindsight it is difficult to see why impulsivity was ever seen as a single construct, but we should not forget that the impulsivity subtypes have considerable overlap as well. First, response inhibition deficits and delay aversion often go together very well (see Impulsivity outside the lab). Second, psychostimulants work very well in patients suffering from ADHD, regardless of their impulsivity types. And finally, there is an important role for serotonin in both response inhibition and delay aversion. Having differentiated the two impulsivity subtypes in the previous paragraphs, we should therefore ask what the similarities are. The aim of this paragraph is to unite the two impulsivity subtypes in a single model. In this model, response inhibition is a reflection of competitions in response selection. Delay aversion is the result of preferences in the reward circuitry, which then primes the response selection circuitry to the desired option (Figure 1).

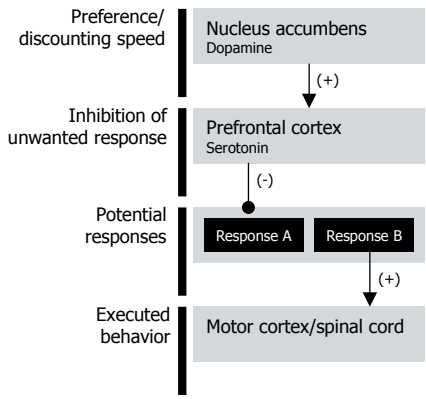

Figure 1 Relationship between neurobiological substrates of response inhibition and delay discounting. Arrows indicate excitatory input, while circles indicate inhibitory input. Dopamine in the motivational areas determines delay-discounting speeds. Motivational areas communicate this preference to the prefrontal cortex, which in turn inhibits the unwanted option. In response inhibition, a serotonin filter similarly inhibits unwanted responses.

Serotonin lesions by local administration of 5,7-DHT causes a failure of response inhibition in several tasks (Harrison et al. 1997; 1999), but does not alter delay discounting speed (Winstanley et al. 2003b). However, in animals with serotonin depletion, D-amphetamine is not as effective in reducing impulsivity as in intact animals (Winstanley et al. 2003b). We tentatively conclude that serotonin is a necessary link in the process of alleviating impulsivity with psychostimulants. We postulate that the process leading to the beneficial effects of psychostimulants on aversion to delayed rewards eventually makes use of a pathway that is also responsible for the effects of serotonergic manipulations on response inhibition. The two types of impulsivity are thus ordered serially in the brain, and the final pathway is mediated by serotonin.

Serotonin appears to fulfill a dual role in this final pathway, mediating both response selections in delay aversion tasks as well as in response inhibition. To unify these roles, we postulate that serotonin is involved in response selection by inhibiting responses unwanted by high-level areas (the prefrontal cortex). In a response conflict, where different parts of the brain favor different actions, higher areas such as the prefrontal cortex, filter possible responses using an inhibitory serotonin signal, thereby stopping unwanted responses and allowing favored actions to be executed. Manipulations to the serotonergic system therefore lead to alterations in the selection of the desired response. This is exactly what is observed after administration of 8-OH-DPAT. Binding of 8-OH-DPAT to the 5-HT_{1A}-autoreceptor causes a decrease in serotonin release, thereby causing a loss of control of the higher areas over response selection. At low dosages, this will cause a more random pattern in choice, as observed by Evenden and Ryan (1999). At higher dosages, control will be completely lost, and lower-level circuitry will determine the choice made, causing a shift to the small but immediate reward (Winstanley et al. 2005).

In this model, dopamine is involved in response preference. If a preference is altered as a result of administration of a psychostimulant, this changed preference leads to alterations in response selection. Therefore, changes in preference cannot result in changes in behavior unless serotonin is present to inhibit the unwanted response. Thus, D-amphetamine cannot attenuate impulsivity in delayed reward tasks in the absence of serotonin. This prediction is confirmed both after depletion as a result of local 5,7-DHT infusion in the brain (Winstanley et al. 2003b), and also after lowering available synaptic serotonin by stimulation of the 5-HT1A-autoreceptor by 8-OH-DPAT (Winstanley et al. 2005).

In tasks measuring response inhibition, such as the stop-signal task, serotonin is also involved in the inhibition of unwanted responses, but in the stop-signal task, a response is unwanted when it is deemed inappropriate as a result of an inhibitory stimulus. Without the inhibitory function of serotonin, the newly processed information cannot result in the appropriate response selection. In such cases, response inhibition fails. In rats, deficits in response inhibition after serotonin depletion are observed in many tasks, such as a go/nogo task (Harrison et al. 1999), the five-choice serial reaction time task (Harrison et al. 1997), extinction (Beninger and Phillips 1979), and certain types of aggressive behavior (Vergnes et al. 1986; Vergnes et al. 1988).

In humans, tryptophan depletion may also lead to deficits in response inhibition (Crean et al. 2002).

Contributions of dopamine and serotonin can never be completely dissociated, as the two systems interact on many levels (Sakaue et al. 2000). In particular from the field of addiction, much is known about the influence of specific serotonergic drugs on the effects of psychostimulants. Local activation of 5-HT_{1B}-receptors in the nucleus accumbens can reduce the effects of D-amphetamine on a conditioning task (Fletcher and Korth 1999). In addition, this manipulation can also reduce responding for local injections of D-amphetamine (Fletcher et al. 2002). The effects of cocaine and GBR-12909 (a dopamine reuptake inhibitor) have been shown to be enhanced by stimulation of 5-HT_{1B}-receptors (Parsons et al. 1996; Przegalinski et al. 2002). Mice lacking 5-HT_{1B}-receptors self-administer more cocaine during operant sessions (Rocha et al. 1998). These data are supportive of a necessary serotonergic link in the realization of the effects of psychostimulants.

One prediction of the serial-process model is that motivational areas in the brain are not required for proper response inhibition. Indeed, rats with lesions to the nucleus accumbens, a structure of vital importance to delay aversion (Cardinal et al. 2001), do not show impaired response inhibition (Eagle and Robbins 2003b). A potential problem with such research is that most animal studies of impulsivity require the animal to respond for food. Lesions of motivational structures may impair food-motivated behavior.

Another prediction made by the model, is that inhibitory areas, like the frontal cortex, do play a role in delayed reward tasks, but only in the modulating effects of psychostimulants. Unfortunately, no studies have been conducted to directly investigate this prediction in delayed reward tasks.

Overview of this thesis

The aim of this thesis is to identify putative drug targets for the treatment of impulsivity. To that end, we set out to find a good animal model for impulsive behavior. The best candidate was the spontaneously hypertensive rat (SHR: Sagvolden et al. 1992; Sagvolden et al. 1993). In Chapter 1, we examined the SHR in two tests for impulsivity: the five-choice serial reaction time task and the differential reinforcement of low-rate responding (72 s) task. The results, especially the effects of methylphenidate, were quite disappointing, and we therefore decided to take another view on modeling in Chapters 2 and 3. In addition, the tasks we used in this first study appeared to be unsuited for a very specific measurement of impulsivity subtypes. Therefore, in Chapter 2, we tested isolation and group-reared animals in the stop-signal task and the delayed reward task. The aims of this experiment were to develop these more specific measurements, to understand the contribution the environment makes to the development of these two types of impulsivity, and to find pharmacological targets for the treatment of these impulsivity subtypes. In Chapter 3, we departed from the standard way of modeling disorders altogether and adopted a new strategy based on endophenotypes. We quantified and correlated measures of response inhibition, delay aversion, locomotion, extinction of learned responses, aggressive and sexual behavior. While the important field of addiction was left out of this study, we conducted a study in human subjects in Chapter 4 examining the relationship between addiction and delay aversion. We conclude that delay aversion may share an underlying mechanism with extinction of conditioned responses, aggressive behavior and addictive behavior. Therefore, in Chapter 5, we tested several drugs not tested before in delayed reward tasks that bind to receptors thought to be important in these behaviors: the dopamine D_3-receptor and the serotonin 5-HT_{1A} and 5-HT_{1B}-receptors may be involved in addiction and aggression, and the NMDA-receptor may be involved in extinction. The dopamine D_3-receptor agonist 7-OH-DPAT increased delay aversion impulsivity, indicating a possible involvement in delay aversion. The 5-$HT_{1A/1B}$-receptor agonist eltoprazine slightly decreased delay aversion impulsivity, and we therefore examined the effects of eltoprazine in Chapter 6 in more detail. In Chapter 6, we test the effects of eltoprazine both on tests for response inhibition and for delay aversion. Much like D-amphetamine, eltoprazine increased choice for the large reward and decreased response inhibition. A microdialysis study indicated that this effect was probably not due to an effect on dopamine transmission. We therefore conclude that the mechanism by which eltoprazine lowers delay aversion impulsivity is unique and may be effective in the treatment of nonresponders to methylphenidate treatment, or in the treatment of impulsivity with comorbid aggression.

Finally, we will discuss the two hypotheses outlined in this thesis. The first hypothesis states that the two impulsivity subtypes are independent from each other, and describes the way in which they are related to other behaviors, including aggression and extinction. The second hypothesis is the serial-process model as outlined above.

Impulsivity outside the lab

Impulsivity outside the lab

This thesis was written to support the theory that impulsivity consists of two separate and very different constructs. The first is an inability to stop planned or ongoing behavior. The second is an aversion to delays to rewards that are not immediately available. The way these constructs are handled in this thesis suggests that the concepts are so very different, that the reader may wonder why it has proven to be so difficult to recognize that impulsivity is not a unitary concept. The answer consists of two parts. First, it is always easier to see things once you know what to look for. As a result, the two concepts are very different in hindsight. Second, in real life situations, impulsive behavior often has elements of both constructs. To illustrate this point, I will discuss two types of real-life impulsivity and identify the two constructs.

Impulsive shopping

Buying something on impulse can attain pathological degrees. Some people are unable to resist spending huge amounts of money. This maladaptive behavior contributes to serious debts. Approximately 3 out of 10 families are frequently or always in debt, and as much as 60% of adolescents between 21 and 25 are in debt. Finally, approximately 200,000 Dutch families are in serious financial trouble, and need help sanitizing their debts (NIBUD 2005).

Are impulsive shoppers unable to resist the thoughts associated with behaviors that lead to the act of buying? They probably are. People with serious debts often can't resist buying some of the items on display near the checkout (often DVDs, or over-priced candy), and feel regret almost immediately after. Do impulsive shoppers not value savings the same way other people do? They probably don't. They might value a reward that is available in the future less then money that can be spent immediately.

Unsafe sex

In 2005, the dangers of having unprotected sex are well known to everybody. Although 85% of Dutch teenagers report using condoms when having sex (Tripp and Viner 2005), this figure may drop to 30% on holidays (Rogstad 2004).

Why do people practice unsafe sex? Again, both types of impulsivity may contribute. First, they may find it difficult to inhibit their behavior in the time leading up to the sexual act. Immediately after having sex, other considerations may gain importance. Another reason people have unprotected sex on impulse may be that the risk of actually contracting a disease (or becoming pregnant) is deemed very low. In other words, the immediate gain is favored over a distant punishment.

Spontaneously hypertensive rats do not predict symptoms of attention-deficit hyperactivity disorder

Pharmacology, Biochemistry and Behavior (Accepted)

Filip van den Bergh
Emilie Bloemarts
Johnny Chan
Lucianne Groenink
Berend Olivier
Ronald Oosting

The validity of the Spontaneously Hypertensive rat (SHR) as a model for Attention Deficit Hyperactivity Disorder (ADHD) is explored by comparing the SHR with Wistar-Kyoto (WKY) and Wistar rats in a number of different tests. In the open field, SHR are hyperactive compared to both Wistar and WKY, but only at specific ages. At those ages, methylphenidate (1 mg/kg) did not attenuate hyperactivity. Subsequently, a dose response study of methylphenidate (0.1-10 mg/kg) was conducted in the Differential Reinforcement of Low-rate responding (DRL)-72s and five-choice serial reaction time tests (5-CSRTT). Compared to WKY but not Wistar rats, SHR performed worse on the DRL-72s. Performance was not improved by methylphenidate (0.1-1.0 mg/kg). In the 5-CSRTT, attentional performance was similar for all rat strains, but Wistar rats made more impulsive responses than both the SHR and the WKY. Methylphenidate only attenuated impulsivity in Wistar rats. Because SHR do not consistently display symptoms of ADHD across the different tests, and methylphenidate effects were observed in both WKY and Wistar rats, but not in SHR, we conclude that SHR is not a representative animal model for ADHD.

Introduction

Attention deficit hyperactivity disorder (ADHD) is a complex CNS disorder characterized by hyperactivity, inattention and impulsivity. The disorder is diagnosed in 4% to 12% of 6- to 12-year old children, and symptoms may persist into adulthood (Resnick, 2005; Wender, 2002; Wolraich et al., 1998). ADHD is usually treated with methylphenidate or comparable mild psychomotor stimulants. Although 70-90% of patients respond to such treatment, there are several drawbacks to using stimulant medication. First, stimulants can induce insomnia, anorexia, headache, and stomach-problems. Second, there is a potential for abuse, and not much is known about long-term effects. And third, methylphenidate has a short half-life, further limiting its use (Bolanos et al., 2003; Goldman et al., 1998). To discover new drug targets involved in processes underlying ADHD, animal models are indispensable. Because the diagnosis of ADHD itself is not without controversy, modeling ADHD in animals is difficult. Nevertheless, different animal models for ADHD have been proposed (Davids et al., 2003), of which the spontaneously hypertensive rat (SHR) seems the best validated model. This rat strain seems to display all three symptoms of ADHD: hyperactivity, an attention deficit, and impulsivity (Sagvolden, 2000). In addition, several biochemical differences between the SHR and the normotensive controls are reminiscent of ADHD. SHR differ from controls in the dopamine system, including an altered response to psychostimulants (Oades, 2002; Russell et al., 1998; Russell et al., 2000b; Volkow et al., 2001), an increased noradrenergic activity (Russell et al., 2000a), and a decreased serotonergic functioning (Kulikov et al., 1997; Nakamura et al., 2001). The use of the SHR as a model for ADHD, however, is not without problems. Although the SHR and WKY were derived from the same colony of outbred Wistar rats, they were derived at different times (WKY after SHR) and therefore the WKY are not exactly the normotensive genetic analog of the SHR (Okamoto, 1969), and an increasing number of studies address the validity of the WKY rat as the control animal for the SHR. The WKY is known for its inactivity, leading to an exaggeration of the ADHD-like symptoms in the SHR. In fact, the WKY is very susceptible to learned helplessness, and has even been proposed as a model for depression (Wieland et al., 1986; Will et al., 2003). When both WKY and SHR are compared to other often-used rat strains, it seems that the SHR is not so much hyperactive, but the WKY is particularly inactive instead (Bull et al., 2000; Pare, 1989; Sagvolden et al., 1993).

The usefulness of the SHR as a model for ADHD, however, is not defined by the resemblance of its behavior to the symptoms of ADHD alone. Predictions about characteristics of ADHD resulting from experiments using the SHR should correspond to findings in the patient population, so the animal model may be used to test putative new medications (Geyer and Markou, 1995). Although necessary, predictive validity is not sufficient for a good animal model. Specifically, methylphenidate, the treatment of choice for patients suffering from ADHD, should have a similar alleviating effect in the SHR and in the patient population (Aron et al., 2003). Although there have been more studies on the effects of methylphenidate in the SHR, only few

have tested the animal model in more widely validated tasks for attention and impulsivity, such as the five-choice serial reaction time task (5-CSRTT), and the differential reinforcement of low-rate responding (DRL) task (see for example Evenden and Meyerson, 1999; Sagvolden et al., 1992b; Sagvolden et al., 1993).

The goal of the present experiment was to assess the validity of the SHR as a model for ADHD in several well-validated tests for activity, attention and impulse control. The frequently used Wistar rat served as a control in addition to the WKY, to counteract the previously described problems associated with the WKY strain as a control strain. Furthermore, to assess the predictive validity of the SHR as a model for ADHD, the effects of methylphenidate on activity, attention and impulsivity have been measured.

Methods

Experimental design

In Experiment 1, Wistar rats were used to establish a methylphenidate dose range using the five-choice serial reaction time task. In Experiment 2, both male and female rats of all three strains were repeatedly tested for locomotor hyperactivity in an open field test at age 30 d, 44 d, 58 d, 72 d, 86 d, and 100 d. In Experiment 3, another group (at age 30-44 d because the previous experiment indicated that the differences between the SHR and the other strains were largest at that age) consisting of all three strains was tested in the open field, now under administration of methylphenidate. In Experiment 4, half of the animals used for Experiment 3 started the acquisition-reversal-extinction battery, used to measure general operant ability of the animals because most tests for attention and impulsivity are operant tasks. Changes in such basic operant behavior may underlie changes found in the performance on other, more complex tasks, such as those used in Experiments 5 and 6. After the acquisition-reversal-extinction battery, rats progressed to Experiment 5, and were trained in the DRL-72 s task, a test that has been in use for over forty years (Richards et al., 1993; Woolverton and Alling, 1999). In this task, animals are rewarded for pressing a lever - but only if the previous response was more than 72 s previously. Experiment 6, the 5-CSRTT, was conducted on the other half of the animals used in experiment three (Carli et al., 1983; Robbins, 2002). In this task, animals are rewarded food pellets for responding to brief flashes of light in one of five holes. The accuracy of responding serves as a measure for attention. Criterion performance for experiments five and six was reached at approximately the same time, and the effects of 0.1, 1 and 10 mg/kg methylphenidate (p.o., 60 min before testing) on DRL and the 5-CSRTT performance were tested.

All operant procedures are available for download at the Medstate Notation Repository (www.mednr.com).

Subjects

The study was composed of six experiments conducted in three separate groups of rats. In the first experiment, 20 male Wistar rats (HsdCpb:WU) obtained from Harlan (The Netherlands) were used for an initial methylphenidate dose-response study. In the remaining experiments three different strains were used: Wistar rats (HsdCpb:WU), Wistar-Kyoto rats (WKY/NHsd), and Spontaneously Hypertensive Rats (SHR/NHsd). In the second experiment, 8 male and 8 female rats of the three strains were tested in the open field at different ages. The rats were bred at the Faculty's animal facility from parents obtained from Harlan (United States). For the third experiment, 16 male rats of each of the three strains were used. The Wistar rats were bred at Harlan's facilities in the Netherlands, while Harlan imported both the 16 SHR as well as the 16 Wistar Kyoto rats from their United States facility. For the fourth, fifth and sixth experiment, the same animals were used as in experiment three.

All animals were housed in groups of four of identical strain in a light (lights on from 7:00 AM to 7:00 PM), temperature (21 ± 2 °C) and humidity (55 ± 5 %) controlled facility. All tests were conducted during the light phase. To motivate the rats (except those used in experiment 2) to respond during the operant tasks, they were on a diet of 15 g of standard laboratory chow per day and were rewarded food pellets for proper responses in the operant tasks. Animal weight corresponded to 85 ± 5% of free-food weight. Water was freely available. The animals were weighed and checked for health problems weekly by a veterinary technician. The ethical committee on animal experiments of the Faculties of Pharmaceutical Sciences, Chemistry and Biology of Utrecht University, The Netherlands, approved the experiments.

Apparatus

The open field used in the second experiment was different from the open field used in the third experiment. In the second experiment, open field tests were conducted in four square gray PVC containers measuring 75 cm by 75 cm and 40 cm tall. In the third experiment, cylinders of 45 cm in diameter and 30 cm tall were used. The movements of the animals in the open field were tracked over 60 min via a video-system in real-time using Noldus Ethovision 3 (Noldus et al., 2001).

Eight Skinner boxes (MED Associates) were used, all equipped with a food magazine, delivering 45 mg Noyes precision pellets Formula P (Research Diets, New Brunswick, USA). The operant chambers were controlled by MED-PC IV software. Four of those eight boxes were used for the acquisition-reversal-extinction tests and the DRL procedure, and were equipped with retractable levers to the left and right of the food magazine. The remaining four boxes were used for the 5-CSRTT, and were equipped with a five-hole nose poke wall opposite to the food magazine. In each of these holes, a light stimulus could be presented, and nose poke responses could be registered. In addition, motion detectors were mounted on the ceiling in these boxes.

Open field

In the second experiment, animals were placed in the open field untreated. In the third experiment, one hour before each open field test, animals (around age 30 d) were either injected with saline or 1 mg/kg methylphenidate (p.o.). The animals remained in the open field for 60 min. After the session, all subjects were returned to their home cage.

Acquisition-reversal-extinction battery

At the start of each acquisition session, both levers were extended into the operant cage. Subjects received a food reward every time they pressed the left lever only. If subjects received 25 pellets in a 30-min session, they reached criterion, and received no more acquisition sessions. Reversal sessions were similar to acquisition sessions, but in these sessions the animals had to press the lever not reinforced in the previous session. All animals received two reversal sessions. During the extinction sessions, both levers were again extended, but pressing the levers yielded no food reward. All animals received five extinction sessions.

DRL-72s

Only animals that were included in the acquisition-reversal-extinction battery were trained on the DRL schedule. Since these animals had just received extinction training, they had to be retrained to press the right lever for food reward. The right lever was the only extended lever during the DRL training. After initial retraining, animals received one-hour DRL training sessions daily. In these sessions, only responses that were more than 6 s after the previous response were rewarded. The minimum inter-response time was increased over the course of the training until the target inter-response time of 72 s had been reached (6 s, 12 s, 24 s, 36 s, 48 s, 60 s, 72 s).

When subjects reached the target inter-response time, they were injected with methylphenidate one hour prior to the test. The dosages (0.1 mg/kg, 1.0 mg/kg and 10 mg/kg) were administered according to a within-subjects Latin-square design. Drug tests were conducted in two consecutive weeks on Tuesday and Thursday.

Five-choice serial reaction time task

In phase 1, animals were habituated to the operant chamber by putting them in the operant chamber for 15 min, while 15 pellets were freely available in the food magazine. Phase 2 habituated animals to the feeder over the course of two 20-min sessions where food was dispensed every 20 s. In phase 3 animals were trained to make nose pokes in one of the lit apertures of the 5-hole wall. During this phase, the same aperture was lit all the time. Animals reached criterion performance if they made more than 50 nose pokes during a 20 min session. Then, the final training phase commenced. Sessions of this type lasted either an hour, or until 100 trials had been initiated. To start a trial, animals were required to make a nose poke in the food magazine. After the variable (5-10 s) inter-trial interval, a randomly selected aperture was lit, and animals were rewarded for responses in that aperture either during the presentation of the stimulus, or

during the limited hold period that followed presentation. The stimulus presentation times were decreased during training (30 s, 10 s, 5 s, 2 s, 1 s), while the limited hold period was fixed at 5 s. Responses into any other aperture than the target or a failure to respond at all were punished by a timeout. During a timeout, the house light was extinguished for 5 s, after which a magazine nose poke initiated the next trial. For the first experiment (the dose-response study) and the first part of the second experiment (the strain comparison), anticipatory responses were punished by timeout. To increase the chances of finding an effect of methylphenidate, anticipatory responses were no longer punished during the second part of the training for the second experiment (Hahn et al., 2002).

If the subjects' performance at the target stimulus duration (1 s) was adequate (%correct>60%, %omissions<15%), animals were injected with methylphenidate one hour prior to the test. The criteria were obtained by inspecting the performance daily until no further improvement was made. The dosages (0.1 mg/kg, 1.0 mg/kg and 10 mg/kg) were administered according to a within-subjects Latin-square design. Drug tests were conducted twice per week on two consecutive weeks on Tuesday and Thursday.

Data reduction and analysis

All data were analyzed using the SPSS General Linear Model procedure. Variables with a not-normal distribution as determined by visual inspection and by the Kolmogorov-Smirnov test were translated using a log-transformation. Significant differences ($p<0.05$) were further explored using Bonferroni corrected post-hoc comparisons. For strain differences, all strains were compared. For drug effects, comparisons to vehicle conditions were made and p-values were multiplied by the number of non-vehicle administrations to correct for multiple comparisons.

In the open field, drug administration and open field session were entered into the general linear model as a between-subjects variable, while the time served as a within-subjects variable. In the DRL and the 5-CSRTT, drug administration was analyzed as a within-subjects variable.

The acquisition-reversal-extinction battery was analyzed in three parts. First, the acquisition times were compared. Then, the number of responses on the reinforced lever compared to the number of non-reinforced responses was compared for the three strains. Finally, the three strains were compared by the number of responses in the five extinction sessions.

Statistical analysis of responding in the DRL was not restricted to the number of obtained rewards. The responses during the session were collected in 6 s bins, allowing for the calculation of the burst ratio (the number of responses made in the 0-6 s after a previous response compared to the total number of responses), the peak area and the peak location. An elaborate description of this method is provided by (Richards et al., 1993).

Correct and incorrect responses in the 5-CSRTT were calculated as a percentage of the total number of trials that ended in nose poke. Anticipatory responses and omissions however, were calculated as a percentage of all initiated trials. Other measures included in the analysis were

activity as measured by the ceiling-mounted motion detectors, number of magazine responses (sometimes taken as a measure for activity) and the latency to collect rewards (sometimes taken as a measure for motivation), make correct responses and to make incorrect responses.

Drugs

To closely simulate clinical administration, methylphenidate hydrochloride derived from Ritalin tablets (Novartis Pharma, Arnhem) were used. Tablets containing 10 mg of methylphenidate were crushed and suspended in gelatin (0.5%)/mannitol (5%). Suspensions were freshly made every morning, and vortexed before every administration. Ground tablets have not been used before in such research, and because ground tablets (including additives) administered orally may have different pharmacokinetic properties due to a slower absorption rate, we conducted a dose-response study to establish an optimal dose range. First, 5 mg/kg, 10 mg/kg and 20 mg/kg were tested followed by a lower range: 0.5 mg/kg, 2.5 mg/kg and 5 mg/kg (p.o., at 60 min before testing). Based on those results, the dose range for the strain comparisons was determined to be 0.1 mg/kg, 1.0 mg/kg and 10 mg/kg. At the highest dose, dopamine levels in the nucleus accumbens have been shown to more than double after an hour (Gerasimov et al., 2000). Although this dose range is not identical to ranges used in other studies (Thai et al., 1999), the pilot study indicated the range to be optimal for oral administration of ground tablets, allowing for the measurement of both stimulating (high dose) and calming (low dose) effects of methylphenidate. Oral administrations were performed by a skilled technician in freely moving animals.

Results

Experiment 1: Methylphenidate in the five-choice serial reaction time task

The effects of oral administration of methylphenidate on premature responding in the 5-CSRTT were dependent on dose (see Figure 1). In the first experiment using high dosages (5, 10, 20 mg/kg), methylphenidate significantly elevated anticipatory responding ($F[3,51]=13.0$, $p<0.005$). This effect was significant at 10 mg/kg and 20 mg/kg ($p<0.05$ and $p<0.001$ respectively). In the second experiment using lower dosages, methylphenidate also significantly altered anticipatory responding ($F[3,8]=5.0$, $p<0.05$). Although this decrease was most pronounced (41%) at 0.5 mg/kg, it did not reach statistical significance. For further experimentation, a dose range was chosen that incorporated both slightly lower and slightly higher dosages (0.1 mg/kg, 1.0 mg/kg), as well as a dose that resulted in an increase of anticipatory responses (10 mg/kg). In addition, it was decided to use an adapted version of the 5-CSRTT would be used to maximize the effects of methylphenidate. If anticipatory responses go unpunished by timeout, the test becomes more sensitive to the effects of psychostimulants (Hahn et al., 2002).

Experiment 2: Effects of gender and age on open field activity

As can be seen in Figure 2, the development of activity was different for the three strains (age ×

strain interaction: F[3.5,195]=3.5, p<0.005). As a result of the complex interactions, not one strain can be said to be more active than the others across the entire 100 days (main effect of strain: F[2,39]=2.9, ns). At age 30 d, however, male SHR are more active than WKY (separate ANOVA: F[2,41]=4.6, p<0.005, SHR vs. WKY: p<0.05). Because of the SHRs hyperactivity at that time, 30 day old animals were also used in Experiment 3. Hyperactivity of the SHR was not found in female rats.

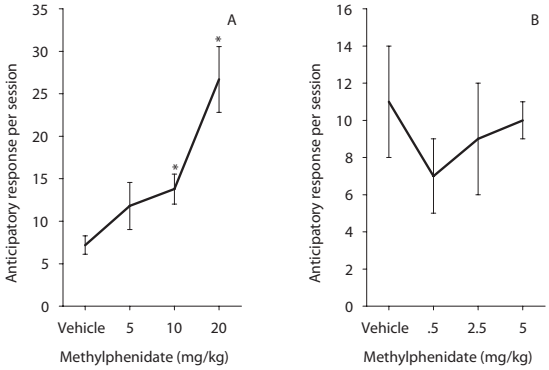

Figure 1 Effects of methylphenidate in two dose ranges on anticipatory responses in the 5CSRTT. Values are cumulative mean responses ± SEM. Significant effects of methylphenidate are indicated with * (p<0.05).

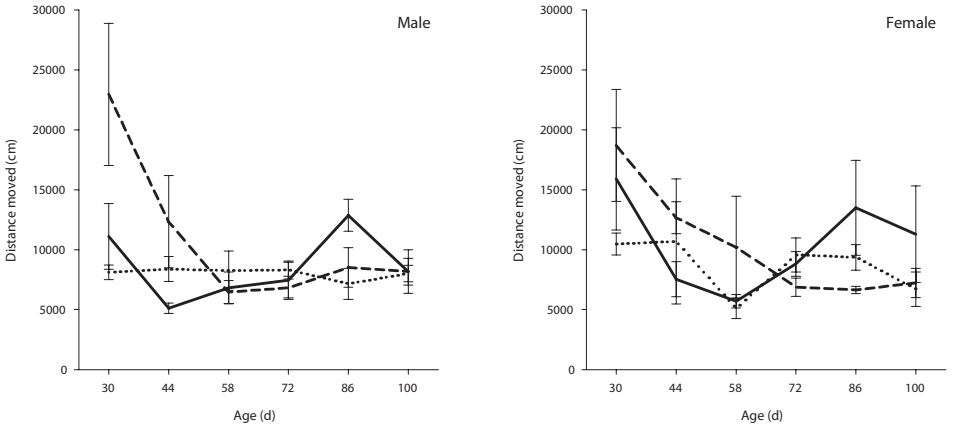

Figure 2 Development of open field activity over six different ages. Distance moved in the open field by male rats is shown in the left graph while activity of female rats is shown in the right graph. Values are total distance moved over 60 m ± SEM. Male SHR are significantly more active at 30 d, while Wistar rats are more active at 86 d.

Experiment 3: Effects of methylphenidate on open field activity

The activity of the three strains on the three consecutive open field tests with and without administration of 1 mg/kg methylphenidate is depicted in Figure 3. Across all three sessions, SHR were significantly more active in the open field than Wistar Kyoto rats (main effect: $F[2,22]=9.5$, $p<0.005$, SHR vs. WKY: $p<0.005$). The difference between WKY and Wistar rats was almost significant ($p=0.055$), whereas activity of SHR and Wistar rats did not differ.

Although the treatment × strain interaction was not significant ($F[2,22]=2.6$, $p=0.1$), treatment effects were calculated separately for each strain. Both the SHR and the normal Wistar rat showed no effect of the 1 mg/kg dose of methylphenidate administration ($F<1$ in both cases). The activity of the WKY rat, however, was suppressed by methylphenidate (treatment main effect $F[1,8]=6.6$, $p<0.05$; treatment´open field session interaction $F[2,7]=4.7$, $p=0.05$). Further exploring this suppression shows that it was particularly apparent in the third open field test (where $F[1,11]=6.2$, $p<0.05$).

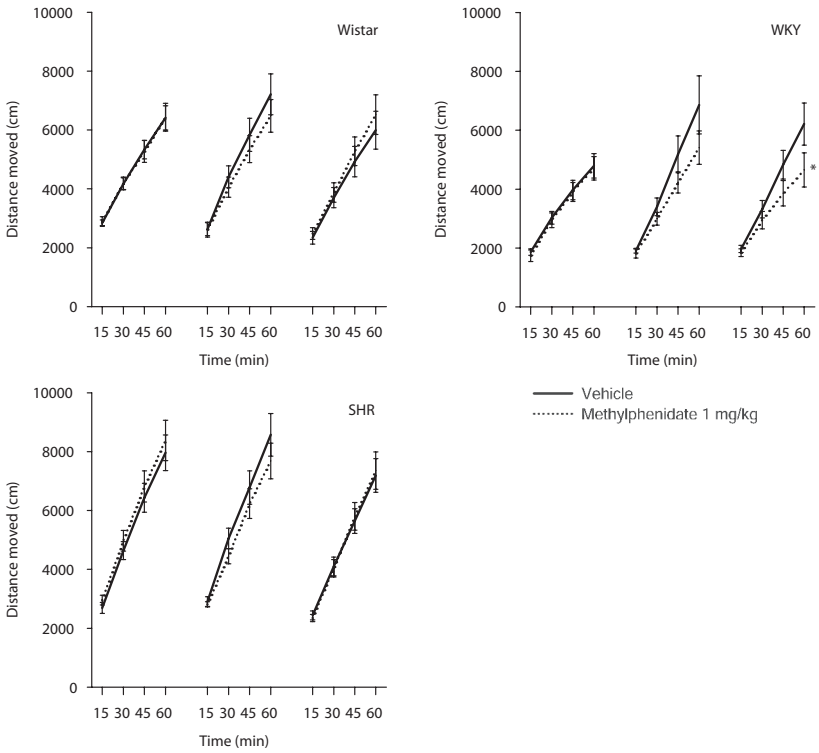

Figure 3 Effects of strain and methylphenidate on cumulative locomotor activity in three consecutive sessions (once per week, starting at age 30 d) of the open field test. Treatments across the three sessions were always the same. Values are cumulative distance ± SEM. Significant effects of methylphenidate are indicated with * ($p<0.05$).

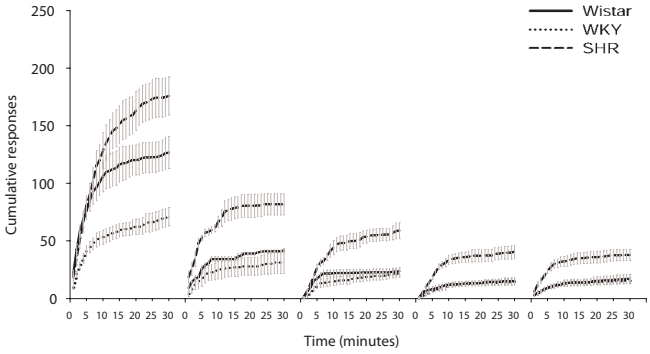

Figure 4 Number of responses during five consecutive extinction sessions. None of the levers yielded rewards. Values are cumulative mean responses ± SEM.

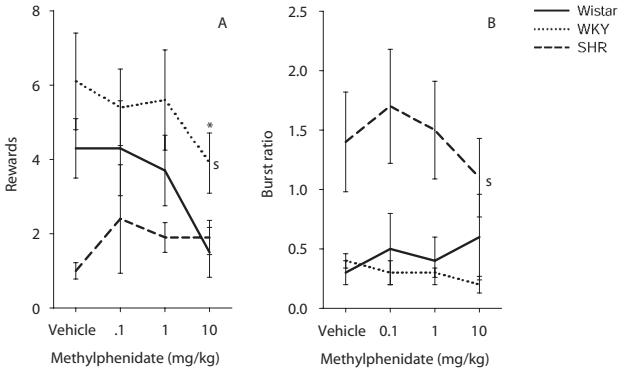

Figure 5 Rewards (A) and burst ratios (B) in the DRL-72 s. Values are group means ± SEM. Significant differences are indicated with s (for a significant strain difference $p<0.05$) and * (for a significant drug effect $p<0.05$).

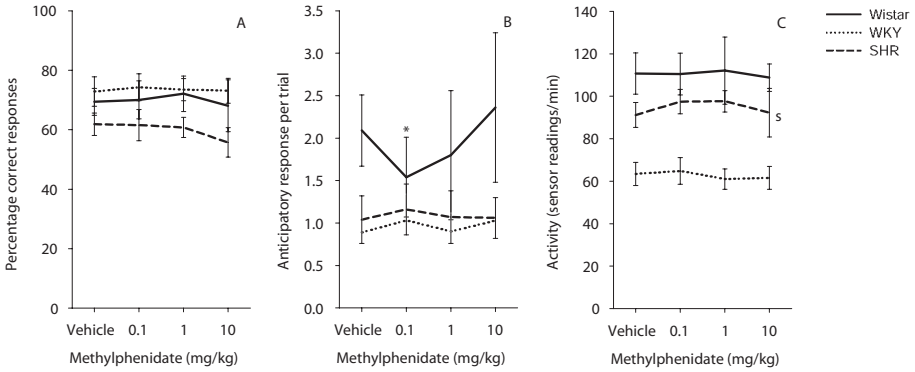

Figure 6 Accuracy (A), anticipatory responses (B) and activity (C) in the five-choice serial reaction time task. Values are group means ± SEM. Significant differences are indicated with **s** (for a significant strain difference $p<0.05$) and * (for a significant drug effect $p<0.05$).

Experiment 4: Acquisition-reversal-extinction battery

The time to acquire lever-pressing did not differ for the three strains (mean±sem: Wistar: 27±6.8 min, WKY: 55±15.2 min, SHR: 38±6.4 min; $F[2,20]=1.8$, NS). The number of responses on the non-reinforced lever during the two reversal sessions was also not different across groups ($F[2,20]=1.2$, NS for the first reversal, $F[2,20]<1$ for the second reversal session). Although the WKY rats lagged behind during the session, the session completion time was not significantly longer ($F[2,20]=2.7$, $p=0.092$). During the five extinction sessions (Figure 4), the SHR responded significantly more than both other strains, and the Wistar rats made significantly more responses than the WKY rats (main effect: $F[2,19]=26.9$, $p<0.001$, SHR vs. WIS: $p<0.001$, SHR vs. WKY: $p<0.001$, WIS vs. WKY: $p<0.05$).

Experiment 5: DRL-72s

The number of rewards obtained and the burst ratio in the DRL is depicted in Figure 5. The other parameters of the DRL (peak latency and peak area) are summarized in Table 1. One Wistar rat was removed from the analysis because it performed too well (26 rewards obtained while the Wistar group mean was 4.3 rewards, Cook's distance was 0.95).

SHR received significantly fewer rewards than WKY rats (main effect: $F[2,18]=5.3$, $p<0.05$, SHR vs. WKY: $p<0.05$). The mean number of rewards obtained by the Wistar rats was lower than the mean for the WKY rats, but higher than the mean for the SHR, resulting in no significant differences. Administration of methylphenidate led to a significant overall decrease in rewards obtained ($F[3,16]=3.6$, $p=0.038$), post hoc tests confirmed that 10 mg/kg methylphenidate resulted in a decrease in rewards obtained compared to the vehicle condition ($p<0.05$).

	Wistar rats			
	vehicle	0.1 mg/kg	1.0 mg/kg	10 mg/kg
Peak latency *d	31.2 ± 8.2	33.9 ± 5.8	32.9 ± 6.0	30.3 ± 5.6
Peak area	0.3 ± 0.1	0.3 ± 0.04	0.3 ± 0.03	0.4 ± 0.1
	Wistar-Kyoto rats			
	vehicle	0.1 mg/kg	1.0 mg/kg	10 mg/kg
Peak latency *d	35.8 ± 3.3	36.1 ± 5.1	38.2 ± 2.7	27.6 ± 2.0
Peak area	0.4 ± 0.03	0.4 ± 0.04	0.4 ± 0.04	0.4 ± 0.03
	Spontaneously hypertensive rats			
	vehicle	0.1 mg/kg	1.0 mg/kg	10 mg/kg
Peak latency *d	23.8 ± 2.1	29.4 ± 3.5	26.4 ± 2.5	21.9 ± 2.5
Peak area	0.3 ± 0.03	0.4 ± 0.04	0.4 ± 0.03	0.3 ± 0.04

Table 1 DRL peak analysis parameters. The table values indicate scores ± standard error of the mean.
*s significant strain effect $p<0.05$, *d significant drug effect $p<0.05$

	Wistar rats			
	vehicle	0.1 mg/kg	1.0 mg/kg	10 mg/kg
Time to complete session (min) *s	24.3 ± 0.6	24.8 ± 0.4	25.3 ± 1.2	24.7 ± 0.6
%Incorrect during stimulus	21.5 ± 4.5	20.9 ± 6.3	18.5 ± 5.4	24.4 ± 7.6
%Incorrect during limited hold	9.1 ± 1.6	9.0 ± 1.2	9.4 ± 1.1	7.5 ± 2.0
%Omissions	5.1 ± 1.1	6.4 ± 2.1	7.0 ± 1.8	7.7 ± 2.6
%Perseveratives	1.4 ± 1.0	1.0 ± 0.5	0.7 ± 0.3	0.9 ± 0.3
Magazine responses	9.4 ± 0.7	9.7 ± 0.9	9.6 ± 1.0	9.3 ± 1.0
	Wistar-Kyoto rats			
	vehicle	0.1 mg/kg	1.0 mg/kg	10 mg/kg
Time to complete session (min) *s	29.8 ± 1.0	36.2 ± 4.0	30.3 ± 2.0	37.2 ± 3.9
%Incorrect during stimulus	17.6 ± 4.2	18.0 ± 5.5	16.8 ± 3.6	17.5 ± 5.1
%Incorrect during limited hold	9.6 ± 1.4	7.7 ± 1.6	9.7 ± 1.0	9.3 ± 1.3
%Omissions	11.6 ± 2.5	12.7 ± 3.1	9.8 ± 2.2	11.1 ± 2.5
%Perseveratives	0.9 ± 0.3	0.9 ± 0.2	1.0 ± 0.4	0.7 ± 0.1
Magazine responses *d	7.3 ± 0.4	5.6 ± 0.8	7.1 ± 0.6	6.0 ± 0.9
	Spontaneously hypertensive rats			
	vehicle	0.1 mg/kg	1.0 mg/kg	10 mg/kg
Time to complete session (min) *s	32.5 ± 1.8	34.1 ± 1.7	33.7 ± 1.2	37.3 ± 3.9
%Incorrect during stimulus	25.7 ± 4.0	27.8 ± 5.2	27.0 ± 4.0	26.1 ± 5.2
%Incorrect during limited hold	12.5 ± 2.9	10.6 ± 1.7	12.2 ± 1.6	18.2 ± 6.5
%Omissions	9.6 ± 3.3	8.6 ± 3.4	9.1 ± 3.6	18.5 ± 9.7
%Perseveratives	2.9 ± 1.3	3.7 ± 1.7	2.5 ± 1.2	2.1 ± 0.8
Magazine responses *d	11.5 ± 0.7	10.6 ± 1.0	9.6 ± 0.8	8.6 ± 1.3

Table 2 Miscellaneous parameters for the five-choice serial reaction time task. The table values indicate scores ± standard error of the mean. *s indicates a significant strain effect $p<0.05$, *d indicates a significant drug effect $p<0.05$

Burst responding was largely dependent on strain ($F[2,18]=7.0$, $p<0.01$). The dose × strain interaction was also significant ($F[6,34]=3.0$, $p<0.05$). SHR show up to 600% higher burst ratios than other strains (SHR vs. WKY: $p<0.01$, SHR vs Wistar: $p<0.05$). Burst ratios of WKY and Wistar rats did not differ significantly. To further explore the dose × strain interaction, the dose effects were analyzed separately for each strain. The effects of methylphenidate on burst responding were strongest in the SHR although the results did not reach significance ($F[3,4]=5.4$, $p=0.069$). There were neither strain nor dose differences on the peak area ($F[2,18]<1$, $F[3,16]<1$, respectively).

The main effect of strain on peak latency did not reach statistical significance ($F[2,18]=3.1$, 0.069), but the peak latency was sensitive to methylphenidate ($F[3,16]=4.4$, $p<0.05$). A post-hoc analysis revealed no differences between dosages.

Experiment 6: Five-choice serial reaction time task

Figure 6 shows the accuracy of responding, the number of anticipatory responses and the activity in the 5-CSRTT. Other five-choice measures are listed in Table 2. WKY rats took significantly longer to reach criterion than both the SHR and the Wistar rats (mean±sem: Wistar: 9±0.4 sessions, WKY: 16±1.9 sessions, SHR: 11±1.0 sessions; main effect: $F[3,21]=7.9$, $p<0.005$, SHR vs. WKY: $p<0.005$, SHR vs. Wistar: $p<0.05$). SHR were about as fast to acquire the task as Wistar rats. Correct responses in the 5-CSRTT did not depend on strain ($F[3,18]<1$). Because the dose × strain interaction bordered significance for the anticipatory responses ($F[3,18]=2.2$, $p=0.061$), the strain differences were calculated only for the vehicle group, and the drug differences were analyzed for each group separately. The main effect of strain was significant ($F[2,20]=4.2$, $p=0.031$), due to the difference between the Wistar rats (the group that scored the most anticipatory responses) and the WKY rats ($p<0.05$). The higher number of anticipatory responses of the Wistar rats did not differ statistically from the level of the SHR ($p=0.088$). The effects of methylphenidate were present only in the Wistar group, where the main effect of dose was significant ($F[3,4]=18$, $p<0.01$). Post hoc comparisons show that 0.1 mg/kg significantly lowered impulsivity compared to vehicle ($p<0.05$). Activity in the operant cage as measured by ceiling-mounted motion detectors was dependent on strain ($F[2,20]=13.2$, $p<0.001$). Both the SHR and the Wistar rats were significantly more active than the WKY rats ($p<0.001$ for both comparisons). The SHR were as active as the Wistar rats ($p=0.11$).

Session duration was dependent on strain ($F[2,20]=18.8$, $p<0.001$). Wistar rats completed the

session faster than both SHR and WKY rats (Wistar vs. SHR: $p<0.001$, Wistar vs. WKY: $p<0.001$). The time to complete the session was not different for SHR and WKY rats. Incorrect responses during stimulus presentation and during the limited hold were not affected by strain ($F[3,18]<1$ for both). The slightly higher level of omissions of the WKY rats was also not significant ($F[3,18]<1$). Because of a tendency towards a dose × strain interaction ($F[6,38]=2.0$, $p=0.089$), the data were analyzed separately. For the SHR and the WKY rats, a decrease of magazine responses was measured with increasing dosages of methylphenidate. This decrease did not reach statistical significance ($F[3,5]=4$, $p=0.085$ and $F[3,5]=3.7$, $p=0.098$ respectively). For the Wistar rats, there was no such pattern ($F[3,4]<1$). The latency to collect obtained rewards, the latency to make a correct response and the latency to make an incorrect response were no different for each of the three groups (main effects: $F[3,18]=1.0$, NS, $F[3,18]<1$, $F[3,18]<1$ respectively).

Discussion

The aim of the present research was to validate the SHR as a model for ADHD. SHR were more active in the open field than WKY rats, but only at specific ages. Furthermore, this activity was not normalized by methylphenidate, only WKY were less active with methylphenidate administration. Acquisition and reversal of lever pressing was not different, but SHR did show slower extinction. SHR made more burst responses and received fewer rewards in the DRL, but methylphenidate did not normalize their performance. SHR did not show an attention deficit in the 5-CSRTT, and were less impulsive than Wistar rats. Only impulsivity of Wistar rats was alleviated by methylphenidate.

The diagnostic and statistical manual (DSM-IV) describes the first symptom, hyperactivity, as an inability to remain seated or keep quiet, and as often on the go. In animals, activity is most frequently measured in the open field. Open field behavior of the three strains has often been studied, although the data are not very consistent. SHR are sometimes more active than control strains (Hard et al., 1985), while sometimes control strains are more active (Ferguson et al., 2003; Sagvolden et al., 1993). This hyperactivity is not always present at the first session (e.g. Knardahl and Sagvolden, 1979), and sometimes psychomotor stimulants suppress this activity (Myers et al., 1982; Wultz et al., 1990). In the present study, only young (30 d old) SHR traveled much more than WKY. At the same age, Wistar rats traveled about as much as the SHR, but the pattern of activity over an hour in the open field is different. Although Wistar rats are as active as SHR in the first fifteen minutes of the test, in the remaining 45 minutes, Wistar rats are about as active as WKY rats. The different origins of the animals cannot account for this difference, as the results of animals bred in our facility (Experiment 2) and imported animals (Experiment 3) are very similar. Why do juvenile SHR keep exploring the open field? Previous studies have shown that SHR do not habituate to novel environments as fast as WKY do (Hendley et al., 1985). Are they, like hippocampally damaged animals, unable to remember

where they have been (Good and Honey, 1997)? Performance of SHR in the Morris water maze suggest they do not have a worse spatial memory, and may even exhibit faster spatial orientation than WKY and Sprague-Dawley rats (Diana, 2002; Ferguson and Cada, 2004). Perhaps the SHR rats are less anxious than the other two strains. Indeed, 74 day old SHR display less anxiety-related behavior in the elevated plus maze (Ferguson and Gray, 2005). Anxious WKY rats may habituate to the open field with repeated exposure, and may therefore also explain why WKY rats display increased locomotor activity in the third session of Experiment 3. Whatever the cause of locomotor hyperactivity in the SHR, this increase is not attenuated by administration of methylphenidate. In contrast, activity of the WKY is attenuated by methylphenidate. Although it would be interesting to study why this hyperactivity disappears over time, the insensitivity to methylphenidate suggests that this process is unrelated to ADHD.

Comparing our results to literature, we conclude that SHR show badly replicable performance in the open field (Ferguson et al., 2003; Hard et al., 1985; Knardahl and Sagvolden, 1979; Myers et al., 1982; Sagvolden et al., 1993; Wultz et al., 1990). Based on replicability and pharmacology, we conclude the SHR in the open field is not a useful model for ADHD.

As a measure of the second symptom of ADHD, an attention deficit, the 5-CSRTT was used, an operant task in which animals are trained to detect brief flashes of light. The accuracy of responding reflects attention, while the ability to withhold responses until a stimulus has been presented measures impulsivity. In line with previous research, SHR perform no different from Wistar controls (De Bruin et al., 2003). WKY rats show a similar level of accuracy, but required more sessions to reach criterion performance. This slower acquisition may be related to the inactivity of the WKY rats, which was also reflected in the activity levels as measured by the motion detectors. Because the SHR were not less accurate, and because methylphenidate did not result in increased attention, SHR in the 5-CSRTT do not seem to be a good model for ADHD. In addition, the present results add to the notion that the WKY rats' inactivity makes it an unsuitable control.

The final symptom of ADHD is impulsivity. Impulsive responses are rapid responses made without much forethought or deliberation (Evenden, 1999). Patients fail to delay their responses to the appropriate moment: they give answers before questions are completed or are unable to wait their turn in games. To test for impulsivity in rats, the DRL and the 5-CSRTT were used.

In the DRL, animals are rewarded food pellets for pressing a lever 72 s after their previous lever response. The SHR and the WKY have been tested in this paradigm before, using the Sprague-Dawley strain as an additional control (Bull et al., 2000). There have not been many reports of the effects of methylphenidate in the DRL, but other psychomotor stimulants generally worsen

performance (Balcells-Olivero et al., 1998; Balcells-Olivero et al., 1997; Sabol et al., 1995). In the present study, SHR received less rewards than the WKY controls, but not compared to the Wistar rats. In accordance with the lower amount of rewards, the peak location for the response distribution curve of the SHR was shifted towards the left. In addition, they made more burst responses than both control groups. These results matched reports of performance of SHR in the DRL (Bull et al., 2000). In addition to that replication, we extended those results by measuring the effects of methylphenidate in all three strains. The first effect of methylphenidate was a decrease in rewards consistent with earlier reports (Seiden et al., 1979). In contrast to both control strains, the performance of SHR was unaffected by methylphenidate, even though it allowed more room for improvement. The second effect of methylphenidate was on burst responses. Unfortunately, no post-hoc comparisons were significant, probably due to the small number of animals. How should we interpret the elevated burst responding of the SHR, and the effects of methylphenidate on these fast responses? Perhaps the same neural mechanism that led to burst responding in the DRL led to persistent responding during extinction sessions in the acquisition-reversal-extinction battery. During the extinction sessions, SHR pressed the non-reinforced levers as much as twice more than the Wistar controls. The link between burst responses in the DRL and extinction is supported by significant correlations (Pearson's correlation coefficient r) between the burst ratio and the number of responses during the five extinction sessions ranging from 0.52 (in the fourth extinction session; $p<0.01$) to 0.81 (in the fifth extinction session; $p<0.01$). Because of the association between extinction and burst responding, the slower extinction is more likely due to motor hyperactivity rather than deficient memory. Slower extinction can experimentally be induced by serotonergic depletion (Beninger and Phillips, 1979), a procedure that can also lead to an increase in burst responses (Jolly et al., 1999). Persistence during the extinction sessions and elevated burst responses in the SHR are probably related to serotonergic hypofunctioning in the striatum (Nakamura et al., 2001).

The 5-CSRTT provided a second measure of impulsivity: the anticipatory responses made before the stimulus was presented. Contrary to the DRL findings, the SHR were not the most impulsive group. Previous research also reported the SHR is no more impulsive than control groups (De Bruin et al., 2003). In addition, in the present experiment, the more impulsive Wistar rats responded to methylphenidate by a decrease in impulsivity, while the other strains did not. The impulsivity-attenuating effect of methylphenidate was in accordance with literature (Bizarro et al., 2004), and more pronounced than the data from the initial methylphenidate dose-response study. The increase of effectiveness of methylphenidate compared to the initial dose-response study may be due to the anticipatory responses not being punished by timeout (Hahn et al., 2002). In the present study, SHR did not display lowered impulsivity at the same dose or any other dose. The cause of this failure may be due to altered methylphenidate metabolism in SHR (in particular a possible faster clearance), but this will remain speculation without further research.

It is unclear why the 5-CSRTT measure for impulsivity is different from the DRL data and the extinction data, although changes made to the 5-CSRTT may be responsible: in the present experiment, anticipatory responses were not punished by timeout. Although this may have increased our chances of finding an effect of methylphenidate, this may also have decreased our chances of finding a strain effect and concordance with the burst ratio. Anticipatory responses in the adapted version of the 5-CSRTT task may be a measure of a different type of impulsivity than the burst responses in the DRL (Evenden, 1999). Performance in the DRL may be more related to motor inhibitory control (Pattij et al., 2003), while impulsive responses in the 5-CSRTT may be a measurement of a more general concept of impulsivity.

In the DRL, the SHR are more impulsive, but this task was not sensitive to methylphenidate. The 5-CSRTT on the other hand, was sensitive to methylphenidate, but did not indicate the SHR as more impulsive. Deciding what is the right test for measuring impulsivity is difficult, but neither aids in distinguishing the usefulness of the SHR as a model for ADHD. Either the 5-CSRTT is chosen and there is no impulsivity effect, or the DRL is chosen and the predictive validity cannot be established.

This research adds to a growing body of work indicating that SHR are not a reliable, nor a readily reproducible, model for ADHD (Bull et al., 2000; Ferguson and Cada, 2003). Several findings indicate that performance of the SHR is very different from performance of WKY controls, but there are major problems with this comparison. When the SHR is compared to Sprague-Dawley rats or to Wistar rats such as in the present research, the WKY appears to be a very inactive and non-impulsive animal compared to other widely used rat strains, and may be unsuited as a control (Bull et al., 2000; Diana, 2002; Pare, 1989; Sagvolden et al., 1993). In addition, the lack of efficacy of methylphenidate at the tested dosages for any of the domains of ADHD further limits the usefulness of the SHR. Because methylphenidate was effective in several other tests and strains in the present research, this lack of efficacy cannot be attributed to the used dosages.

There is some evidence that SHR can be divided into two subpopulations, one impulsive and one normal (Adriani et al., 2003). The division into subgroups was not tested extensively, however, as the resulting subgroups were very small. Therefore, before accepting that hypothesis much more research is necessary to make sure that this distinction is not an artifact. Furthermore, the effects of psychostimulants in delayed reward paradigms (a shift towards the large but delayed reward) found in the SHR (Adriani and Laviola, 2004) is not specific to that strain (Cardinal et al., 2000). This raises more questions about the usefulness of the SHR as a model.

Although we conclude that the SHR is not a good model for ADHD, this may not be the result of the specific strains used, but rather a more fundamental problem of using two inbred strains as a model for psychiatric illness. A comparison between two strains will often result in a difference on several behavioral measures. Sometimes these differences are valued as to represent a human condition. The number of modeled symptoms is often used as a criterion of model

quality (for example, see Sagvolden, 2000), but each of these traits may be caused by a different mechanism. For example, in the case of the SHR, the genetic cause of hypertension is unrelated to inattentiveness, impulsivity or hyperactivity (Hendley, 2000; Sagvolden et al., 1992a). The co-occurrence of hypertension with impulsivity is therefore not predictive of ADHD. Likewise, all other differences between SHR and WKY may be attributed to separate genetic origins as well, as SHR and WKY are known to be very different genetically (Festing and Bender, 1984; St Lezin et al., 1992).

Development of delay aversion and response inhibition deficits: effects of rearing conditions and selected psychoactive drugs

Filip van den Bergh
Climmy Cruijen
Emilie Bloemarts
Lucianne Groenink
Leon Kenemans
Berend Olivier
Ronald Oosting

Response inhibition deficits and delay aversion may both lead to impulsive behavior. Differences and similarities between these impulsivity subtypes are important for the treatment of impulsivity. We studied the life-span development and induction by social isolation rearing of both impulsivity subtypes in rats using a stop-signal task and a delayed reward task. The effects of D-amphetamine, fluvoxamine and maprotiline were examined. Analysis of performance in both tasks over the life span showed that response inhibition was more stable than delay aversion. Isolation-reared animals displayed a consistent response inhibition deficit compared to controls. Delay aversion in isolation-reared animals compared to controls developed after six months of age. D-amphetamine significantly decreased delay aversion, but had adverse effects on response inhibition. The effects of D-amphetamine on delay aversion were stronger in isolation-reared rats. Fluvoxamine had only minor effects hinting on a subtle involvement of serotonin in both tasks. Maprotiline had no effects. We conclude that pharmacotherapy should consider the impulsivity subtype observed in patients.

Introduction

Impulsivity is a key symptom of many psychiatric disorders, such as attention-deficit hyperactivity disorder (ADHD), mania and addiction. Many studies suggest impulsivity is composed of two different subtypes: response inhibition deficits and delay aversion (Dalen et al. 2004; Evenden 1999; Nigg et al. 2004; Solanto et al. 2001; Sonuga-Barke 2004). Response inhibition is the ability to inhibit planned or ongoing motor routines, or a failure to protect planned or ongoing behavior from outside interference (Barkley 1999). Deficits in response inhibition have been found in both children (Overtoom et al. 2002; Scheres et al. 2004) and adults (Aron et al. 2003; Wodushek and Neumann 2003) suffering from ADHD. The second impulsivity subtype is delay aversion, resulting in a preference for immediate over delayed rewards (Ainslie 1975; Barkley et al. 2001; Solanto et al. 2001; Sonuga-Barke et al. 1992).

While impulsivity (as a symptom of ADHD) is treated as a unitary construct in the Diagnostic and Statistical Manual (DSM-IV 2000), response inhibition deficits and delay aversion differ in a number of important dimensions. Imaging and lesion studies suggest different brain areas underlie delay aversion and response inhibition. In particular, the frontal cortex plays an important role in response inhibition (Aron and Poldrack 2005; Band and van Boxtel 1999; Casey et al. 1997), while tolerance to delayed rewards is mediated by reward circuitry in the brain, such as the nucleus accumbens (Cardinal et al. 2001). In spite of these important differences, impulsivity as a symptom of ADHD is successfully treated with methylphenidate or D-amphetamine regardless of impulsivity subtype (Elia et al. 1991). Both psychostimulants are also very effective in the laboratory: in human and rat tasks for delay aversion (Cardinal et al. 2000; de Wit et al. 2002; Pietras et al. 2003; van Gaalen et al. 2005) and in human response inhibition tasks (Aron et al. 2003; Scheres et al. 2003; Tannock et al. 1989). However, both methylphenidate and D-amphetamine inhibit the dopamine transporter (DAT), as well as the noradrenaline and serotonin transporters (Gatley et al. 1996). These properties of psychostimulants make them very effective as treatments for subtype-agnostic impulsivity. Simultaneously, these properties may induce unwanted and unnecessary side effects if the transporter of a neurotransmitter uninvolved in the patient's actual impulsivity subtype is inhibited. Several lines of evidence suggest that different neurotransmitters are involved in response inhibition and delay aversion, despite the beneficial effects psychostimulants have in both impulsivity subtypes. As an example, the selective DAT antagonist GBR12909 decreases delay aversion (van Gaalen et al. 2005), indicating an involvement of dopamine. In a human response inhibition task, the dopamine precursor L-dopa is not effective, while the tricyclic antidepressant desipramine is (Overtoom et al. 2003), suggesting that the effects of psychostimulants on response inhibition may be the result of noradrenalin or serotonin reuptake inhibition.

Another important dimension in which response inhibition and delay aversion may differ is their induction by environmental versus genetic factors. One strategy to uncover the involvement of environmental factors in the development of impulsivity subtypes is by using social

isolation rearing (Hellemans et al. 2005). Isolation rearing is a potent manipulation leading to a number of significant alterations (Bakshi and Geyer 1999; Lapiz et al. 2003). Of importance to the current discussion of impulsivity, isolation-reared rats display increased response inhibition deficits in a five-choice serial reaction time task under specific conditions (Dalley et al. 2002), but not in a go/nogo task (Hellemans et al. 2005). Delay aversion is decreased in isolated animals, but this difference disappears over time (Hellemans et al. 2005).

The aim of the present study is to further explore the similarities and differences between delay aversion and deficits of response inhibition. We are interested in the development of both subtypes of impulsivity, and the effects of social isolation as a potent environmental factor. Therefore, rats were housed socially or individually from weaning, and tested in operant tasks for both impulsivity subtypes. Delay aversion was measured using a delayed reward task (Cardinal et al. 2001) and response inhibition was measured in the stop-signal task (Eagle and Robbins 2003b). The involvement of dopamine, serotonin and noradrenalin was explored using D-amphetamine, fluvoxamine and maprotiline, respectively.

Methods and materials

Experimental design

Animals from socially and isolation reared conditions were balanced across time and operant boxes. Training for the delayed reward task took approximately two months, while training for the stop-signal task was completed after three months. The computer programs used for operant conditioning can be downloaded from the Medstate Notation Repository (www.mednr.com). The experiments started when both groups reached criterion in the training. The stop task and delayed reward task were performed at the same time to allow for direct comparisons of the used manipulations.

Subjects

Fifty-six male rats (Wistar) arriving on PND 21 were obtained from Harlan (The Netherlands). On arrival, 32 animals were assigned to the stop-signal task, and 24 animals to the delay aversion task. Half of the animals of both groups were housed in groups of four, the other half were housed individually. All animals were kept in a light (lights on from 7:00 AM to 7:00 PM), temperature (21 ± 2°C), and humidity (55 ± 5%) controlled facility. All tests were conducted during the light phase. Rats were on a diet of 15 g of standard laboratory chow per day throughout the experiment, corresponding to approximately 85% of the amount eaten under free-feeding conditions. Water was freely available. The animals were weighed and checked for health problems weekly. The ethical committee on animal experiments of the Faculties of Pharmaceutical Sciences, Chemistry and Biology of Utrecht University approved the experiments.

Apparatus

Open field tests were conducted in four square, gray PVC fields (70 cm L × 70 cm W × 45 cm H). Movements of animals in these 4 fields were tracked over 15 min (5 samples/s) via a video-system in real-time using Noldus Ethovision 3 (Noldus et al. 2001).

Sixteen operant chambers (MED Associates) controlled by MED-PC IV software were used. All boxes were equipped with a houselight and a central food magazine in which 45 mg Noyes precision pellets (formula P) were delivered. Eight extra tall boxes (30 cm L × 24 cm W × 29 cm H) were used for the delayed reward task and were equipped with a curved five-hole response wall opposite to the food magazine. In each of these five holes, a light stimulus could be presented, and nose poke responses could be registered. The left- and rightmost holes were not used in the delayed reward task. The eight standard height boxes (30 cm L × 24 cm W × 21 cm H) used for the stop task were equipped with retractable levers to the left and right of the food magazine, and signal lamps over the levers and the food magazine.

Open field test

All 56 animals were tested for 15 min in the open field for locomotor hyperactivity. The test was conducted after completion of the training for the two operant tasks. Traveled distances were calculated per minute.

Delayed reward

The delayed reward procedure was adapted from Cardinal and coworkers (2000). Animals were placed in a chamber until they had completed all trials (with a maximum of 90 min). The sessions consisted of six blocks of eight trials. Of these eight trials, the first two trials were forced trials and the remaining choice trials. All trials started with the illumination of the central nosepoke hole. During forced trials, responding into the central hole resulted in the illumination of either the left or the right nosepoke hole, determined randomly. A response made into the illuminated hole resulted in delivery of a reward. All other responses were ignored. A response in the left hole resulted in the delivery of a small, immediate reward, while a response in the right hole resulted, after a delay, in the delivery of a large (4 pellet) reward. The nosepoke hole not illuminated in the first trial was always illuminated during the second. After a response, the light in the nosepoke hole was turned off, and the inter-trial interval commenced. The length of the inter-trial interval was variable so that each trial, regardless of the delay to the reward, was equally long. Choice trials were similar to forced trials, except that both the left and the right nosepoke holes were illuminated, and animals had to make a choice for either the small, immediate reward or the large, delayed reward. The delay to obtain the large reward was increased after every block (0 s, 5 s, 10 s, 20 s, 30 s, 60 s).

Training for the task described above consisted of four phases. Phase 1 consisted of 2 sessions of 15 min each. During these sessions, animals received a food reward each time they made a nosepoke in the centre nosepoke hole, which was illuminated. Phases 2 through 4 were similar

to the final task, except different delays to the large reward were used (0/1/3/5/10/20 s, 0/3/5/10/20/30 s, and 0/5/10/20/30/60 s).

Stop-signal task

The procedure used was adapted from Eagle and Robbins (2003a). Animals were placed in a chamber until they had completed 200 trials (with a maximum of 60 min). The sessions consisted of a number of blocks, which consisted of several successful go-trials (1 to 3, determined randomly), and a concluding stop-trial. With the extension of levers or deliveries of food rewards, a signal light above the lever or feeder tray was illuminated to draw attention to the event.

At the start of a go-trial, the left lever was extended for a maximum of 60 s. A response on this lever resulted in the extension of the right lever. The right lever was present for a limited amount of time (the limited hold period), during which the animal had to make a response to receive a food reward. If the animal failed to respond within the limited hold period, an omission was scored, and the animal received a timeout. Stop-trials were similar to go-trials, except for a 400 ms tone that was presented immediately after a response on the first lever, or 800, 700, 600, or 500 ms before the expected response on the second lever (based on previous sessions for each animal individually, see next paragraph). On stop-trials, animals had to inhibit their response on the second lever for the entire limited hold period to receive a food reward. Failure to do so resulted in a timeout. During timeouts, the houselight was turned off for 5 s. After the timeout period, the inter-trial interval commenced automatically. The inter-trial interval was set at 5 s.

The duration of the limited hold period was determined for rats individually, and was based on their previous performance. The limited hold period was defined as the maximum mean time between pressing the left lever and the right lever plus 150 to 300 ms, and ranged between 850 ms and 1500 ms. Stop-signal delays were presented relative to this period (see also previous paragraph).

Training for the task described above consisted of four phases. Phase 1 consisted of 2 sessions of 15 min each. During this session, animals received a food reward each time they made a nosepoke in the feeder tray while the signal light above the feeder tray was on. If animals failed to respond, they received a timeout period. Phase 2 consisted of 2 sessions of 30 min each. In these sessions, the left lever was extended for a maximum of 60 s. Pressing the left lever resulted in a food reward. A failure to press the left lever in time resulted in a timeout period. Phase 3 consisted only of go-trials as described above. In the first phase 3 session, the limited hold period was set at 60 s for all rats. In consecutive sessions, the limited hold period was set at the mean reaction time counted from the response on the first lever to the response on the second lever added to 300 to 400 ms. In phase 4, the stop-trials were introduced in sessions of 200 trials (with a maximum duration of 60 min). The limited hold values for stop-trials started at 0.3 s, and was gradually increased to equal the limited hold value for go-trials.

Data reduction and analysis

All data were analyzed using the SPSS independent sample T-test and general linear model procedures. Variables with a not-normal distribution as determined by visual inspection and by the Kolmogorov-Smirnov test were log-transformed. Within-subjects tests were corrected using Huyn-Feldt correction if the data did not meet the sphericity demands. Significant differences ($p<0.05$) were further explored using Bonferroni corrected post-hoc comparisons between vehicle and each of the three dosages (in effect setting α at 1.67% for the post-hoc tests).

To analyze data from the open field, the total distance moved was calculated, and the data was analyzed using an independent samples t-test. The delayed reward task yielded two different measures. First, the raw choice data was plotted and analyzed using the delay to the large reward as a within-subjects factor. Choices at 0 s and 60 s delay are usually similar for all dosages due to ceiling and floor effects. Therefore, we calculated a second measure: the area under the preference curve (AUPC). This area reflects a theory-neutral measure of delay aversion (Myerson et al. 2001). Low delay aversion leads to a high AUPC.

The stop-signal task yielded four measures. First are the proportion successful go- and stop-trials. Success in stop-trials was corrected for the estimated number of trials the animals were not going to respond, based on the number of successful go-trials. The formula used (Equation 1) was taken from Tannock et al. (Tannock et al. 1989). In addition, the response speed in go-trials (mRT) was registered. The stop-signal reaction time (SSRT) was calculated by taking a percentile based on stop-trial failure of the mRT distribution. This value was then corrected for the onset of the stop-signal (Eagle and Robbins 2003b; Logan 1994).

Baseline differences between animals were calculated immediately after completion of training. In addition, delayed reward and stop task parameters were calculated across the animal life span.

Drugs

All drugs were dissolved in saline and administered subcutaneously (2 ml/kg) 30 min before testing. D-amphetamine sulphate (0.25, 0.5 and 1 mg/kg) and maprotiline hydrochloride (5 mg/kg) were obtained from Sigma-Aldrich, Zwijndrecht, The Netherlands. Fluvoxamine maleate (1, 3, 10 mg/kg) was obtained from Solvay Pharmaceuticals, Weesp, The Netherlands.

$$P_i' = \frac{P_i - (P_o \times P_i)}{1 - (P_o \times P_i)}$$

Equation 1 Method used to correct the raw inhibition probability for omissions made during go-trials. P_i' is the corrected inhibition probability, P_i is the raw inhibition probability in stop trials, and P_o is probability of omissions in go-trials.

Results

Experiment 1: Baseline differences

The group differences after completion of training for the delayed reward and the stop task are listed in Table 1. Isolation-reared animals traveled 25% more in 15 minutes than socially reared animals ($t[49]=2.63$, $p<0.05$). This difference was present throughout the entire session. This finding replicates literature, indicating isolation rearing procedure was successful (e.g. Domeney and Feldon 1998; Einon and Morgan 1978; Gentsch et al. 1988; Hall et al. 1998). Delay aversion was very similar in both groups. This lack of effect was present both in the raw choice data (delay × rearing interaction: $F[5, 110]=0.5$, NS) and in the AUPC ($t[22]=-0.7$,

Measure	Social		Isolated	
Open field				
Distance moved (cm) *	4501	± 273	5667	± 359
Delayed reward task				
Area under the preference curve	31.2	± 3.8	28.0	± 2.5
Choice for large reward at a delay of (%):				
0 s	81	± 8	86	± 3
5 s	79	± 4	83	± 8
10 s	72	± 9	69	± 6
20 s	60	± 11	53	± 8
30 s	51	± 9	40	± 6
60 s	24	± 8	21	± 6
Stop-signal task				
Go response time (mRT; s)	0.91	± 0.05	0.84	± 0.09
Stop response time (SSRT; s) *	0.43	± 0.02	0.50	± 0.02
Go trial success (%)	72	± 2	68	± 3
Stop trial success (%) *	75	± 6	53	± 6
Stop trial success at a signal onset of (%):				
Immediate (zero-delay)	81	± 8	72	± 7
-800 ms	79	± 7	65	± 9
-700 ms	74	± 8	48	± 6
-600 ms	74	± 6	47	± 7
-500 ms	67	± 8	48	± 8

Table 1 Effects of isolation rearing on various parameters measured immediately after completion of training (for measurements over the life span, see Figure 1). Values represent means ± standard errors. Significant effects of isolation rearing are indicated with *.

NS). Finally, isolated animals performed significantly worse than socially reared animals in the stop task, displaying a selective deficit in response inhibition. Go-trial success and speed were similar for both groups (t[29]=1.1, NS and t[29]=0.7, NS, respectively). Corrected stop trial success and SSRT, however, were both impaired in isolated animals (t[29]=2.4, p=0.022 and t[26]=-2.1, p=0.045).

Previous research by Hellemans and colleagues (Hellemans et al. 2005) found effects of isolation rearing on delay aversion, but not on a go/no-go task used to measure response inhibition. In that study, isolation-reared rats were more likely to choose the large reward compared to controls, at least during the initial phases of their study. Because of the discrepancy between their data and ours, we plotted the performance of rats across their entire life span starting from the session that the final task parameters were introduced (the final delay series for the delayed

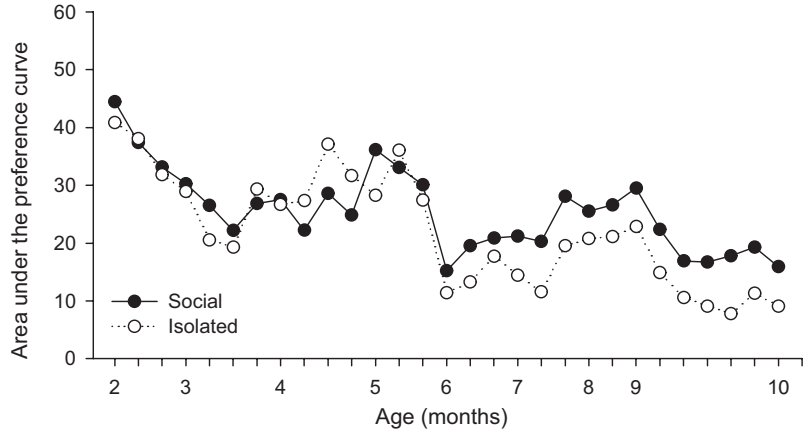

Figure 1A Development of the area under the preference curve of the delayed reward task in socially and isolation reared rats. Values represent means ± standard errors.

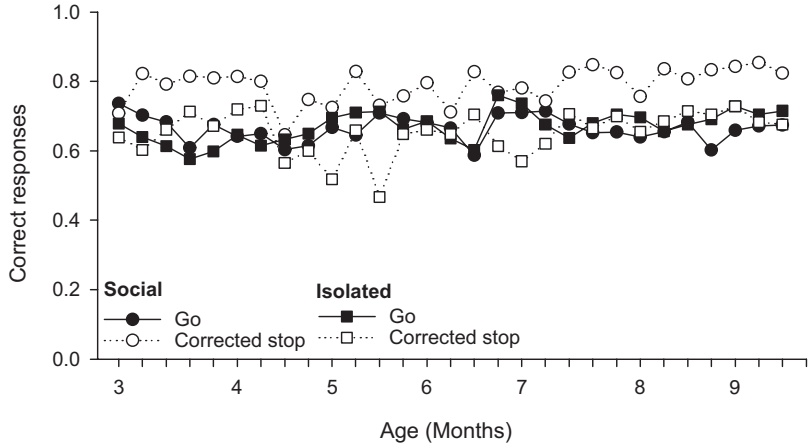

Figure 1B Development of go trial success and corrected stop trial success in the stop-signal task in socially and isolation reared rats. Values represent means ± standard errors.

reward task and the final limited hold times for the stop task). The data are shown in Figure 1, in which each data point represents a selected session. The development of delay aversion is depicted in Figure 1A. While no differences between isolated and socially reared animals were present immediately after training (as reported in Table 1), a consistent difference appeared after age 6 months. After this age, isolation reared animals displayed more delay aversion that socially housed controls. The graph displays several distinct drops (in particular at the start, at 6 months, and at 9 months), which may have been caused by training stops during the year. The development of go- and corrected stop-trial success is depicted in Figure 1B. As can be seen from that picture, socially housed rats consistently displayed higher stop-trial success, while go-trial success did not differ. From Figure 1A and 1B, we can also see that performance in the delayed reward task was much less stable over the life span than performance in the stop-signal task.

Experiment 2: Effects of D-amphetamine

The effects of the psychostimulant D-amphetamine on preference for the large reward are depicted in Figure 2A. A significant 3-way interaction between dose, delay and rearing complicates interpretation of the preference data ($F[15, 135]=2.0$, $p=0.023$). Splitting the data across the rearing conditions showed this interaction was partly caused by the increase in choice for the large reward found in isolated animals after administration of D-amphetamine ($F[3, 12]=4.6$, $p=0.023$), an effect not present in socially reared animals ($F[3, 15]=0.4$, NS). The

D-amphetamine	**Social**		**Isolated**	
Vehicle	18.7	± 2.7	22.2	± 3.7
0.25	21.3	± 3.7	35.0	± 3.2 *
0.5	29.4	± 4.0 *	37.6	± 4.2 *
1	27.2	± 5.1	44.5	± 2.5 *
Fluvoxamine	**Social**		**Isolated**	
Vehicle	18.3	± 2.6	14.4	± 2.5
1	19.4	± 2.7	14.6	± 2.2
3	17.6	± 2.5	12.4	± 1.8
10	24.6	± 3.3 *	16.1	± 3.2
Maprotiline	**Social**		**Isolated**	
Vehicle	20.9	± 4.8	10.9	± 1.3
1.25	13.7	± 2.4	11.3	± 2.8
2.5	19.2	± 2.8	8.8	± 1.1
5	17.3	± 3.1	10.0	± 1.2

Table 2A Effects of various drugs on the area under the preference curve of the delayed reward task in socially and isolation reared rats. Values represent means ± standard errors. Significant differences are indicated with *.

D-amphetamine	Social				Isolated			
	mRT		SSRT		mRT		SSRT	
Vehicle	0.91	± 0.08	0.46	± 0.03	1.04	± 0.08	0.57	± 0.04
0.25	1.06	± 0.07	0.49	± 0.03	1.03	± 0.08	0.58	± 0.03
0.5	1.07	± 0.07	0.55	± 0.03	1.01	± 0.06	0.58	± 0.03
1	0.83	± 0.09	0.54	± 0.05	0.88	± 0.09	0.59	± 0.02
Fluvoxamine	**Social**				**Isolated**			
	mRT		**SSRT**		**mRT**		**SSRT**	
Vehicle	0.96	± 0.06	0.49	± 0.04	0.94	± 0.08	0.57	± 0.04
1	1.02	± 0.05	0.47	± 0.03	0.99	± 0.06	0.53	± 0.03
3	0.85	± 0.07	0.53	± 0.03	0.89	± 0.11	0.56	± 0.03
10	0.88	± 0.07	0.54	± 0.03	0.85	± 0.10	0.57	± 0.04
Maprotiline	**Social**				**Isolated**			
	mRT		**SSRT**		**mRT**		**SSRT**	
Vehicle	1.13	± 0.07	0.47	± 0.06	0.96	± 0.13	0.55	± 0.03
1.25	1.14	± 0.15	0.47	± 0.07	1.19	± 0.11	0.55	± 0.06
2.5	0.89	± 0.08	0.45	± 0.06	1.13	± 0.06	0.59	± 0.05
5	0.86	± 0.11	0.57	± 0.06	1.08	± 0.09	0.49	± 0.07

Table 2B Effects of various drugs on mean go (mRT) and stop (SSRT) response times in socially and isolation reared rats. Values represent means ± standard errors.

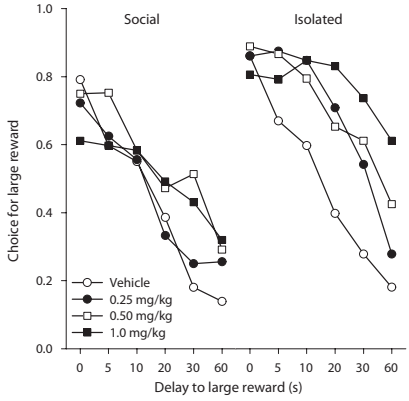

Figure 2A Effects of d-amphetamine in socially and isolation reared animals in the delayed reward task.

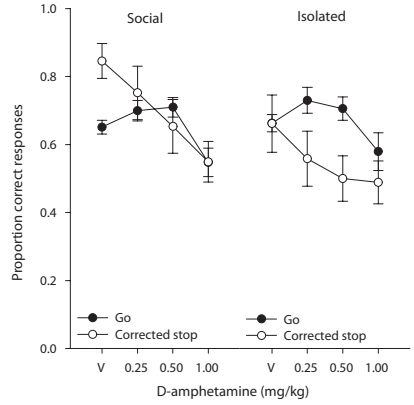

Figure 2B Effects of d-amphetamine in socially and isolation reared animals in the stop-signal task.

AUPC for each dose is listed in Table 2A. The AUPC was more sensitive to the effects of D-amphetamine than the raw preference data ($F[3,63]=9.7$, $p<0.001$). Splitting the isolation and socially reared animals into separate analyses also indicated that the effects of D-amphetamine on delay aversion were stronger in isolation ($F[3,33]=9.5$, $p<0.001$) than in socially reared animals ($F[3,30]=2.7$, $p=0.06$). Compared to vehicle, all dosages lowered delay aversion in isolation-reared animals (0.25 mg/kg: $p<0.05$, 0.5 mg/kg: $p<0.05$, 1 mg/kg: $p<0.005$), while only 0.5 mg/kg D-amphetamine showed a trend to enhance inhibition in the socially reared animals (0.25 mg/kg: ns, 0.5 mg/kg: $p=0.07$, 1 mg/kg: ns).

The effects of D-amphetamine on successful go- and stop-trials are shown in Figure 2B. At the highest dose of 1 mg/kg, D-amphetamine significantly decreased success in go-trials ($F[3, 84]=13.7$, $p<0.001$; Vehicle vs. 1 mg/kg: $p=0.01$). All dosages significantly worsened corrected stop-trial success ($F[3,84]=9.1$, $p<0.001$; Vehicle vs. all dosages: $p<0.001$). While the effects of D-amphetamine on the mean go reaction time (mRT) was significant ($F[2.6, 74]=5.6$, $p=0.03$), none of the dosages differed significantly from vehicle. The effects of D-amphetamine on stop-signal reaction time (SSRT) were not significant ($F[2.6, 68.8]=1.7$, NS).

Experiment 3: Effects of fluvoxamine

The effects of administration of the selective serotonin reuptake inhibitor fluvoxamine on choice for the large reward (Figure 3A) just reached statistical significance ($F[3, 63]=2.8$, $p=0.047$). Post-hoc tests show that none of the dosages had a significant effect compared to vehicle. While the preference curve may suggest that the effect of fluvoxamine is more pronounced in the socially reared animals, this effect is not significant in a separate ANOVA. Analysis of the AUPC (Table 2A) reveals a small decrease in delay aversion after administration of fluvoxamine, but

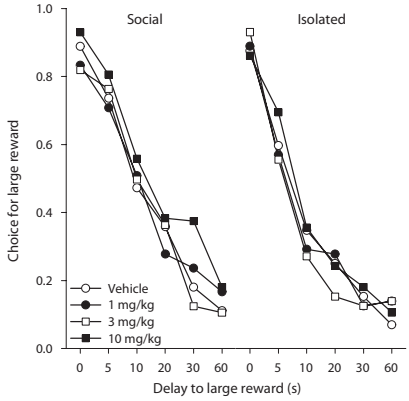

Figure 3A Effects of fluvoxamine in socially and isolation reared animals in the delayed reward task.

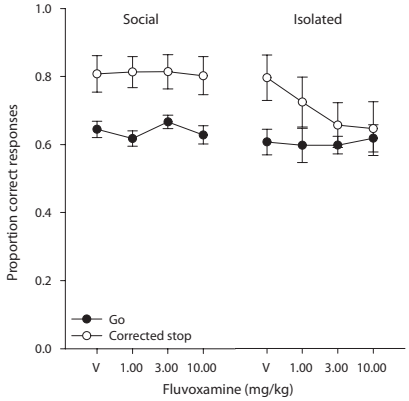

Figure 3B Effects of fluvoxamine in socially and isolation reared animals in the stop-signal task.

only in the socially reared animals (F[3,33]=2.8, p=0.05; Vehicle vs. 10 mg/kg: p=0.033).

As can be seen in Figure 3B, fluvoxamine had no effects on go-trial success (F[2.2, 60.3]=0.3, NS) or corrected stop-trial success (F[3, 81]=2.4, p=0.075). While corrected stop-trial success seems to decrease in the isolation reared animals, this effect was not significant in a separate analysis (F[3, 36]=2.5, p=0.075). The response time measures are again listed in Table 2B. The mRT was not significantly affected by fluvoxamine (F[3, 81]=1.7, NS). The SSRT was significantly increased with 10 mg/kg fluvoxamine administration (F[3, 81]=2.7, p=0.049; Vehicle vs. 10 mg/kg: p=0.015).

Experiment 4: Effects of maprotiline

The selective noradrenalin reuptake inhibitor maprotiline had no effects on choice for the large reward in the delayed reward task (Figure 5A; F[1,16]=1.2, NS). Analysis of the AUPC yielded similar results (F[1, 16]=1.0, NS). Maprotiline had no effects on response inhibition (Figure 5B). Neither the success rates (go-trials: F[1, 18]=0.6, NS; stop-trials: F[1, 18]=0.7, NS) nor the speeds (mRT: F[1, 18]=1.5, NS; SSRT: F[1, 18]=0.3, NS) were significantly altered.

The tests with maprotiline were prematurely cancelled as the preliminary analysis revealed that maprotiline had no effects on impulsivity, and large sores began to develop at the (s.c.) injection sites. Although all animals were included in the graph, a full within-subjects analysis could only be performed on the data for vehicle and 5 mg/kg administration.

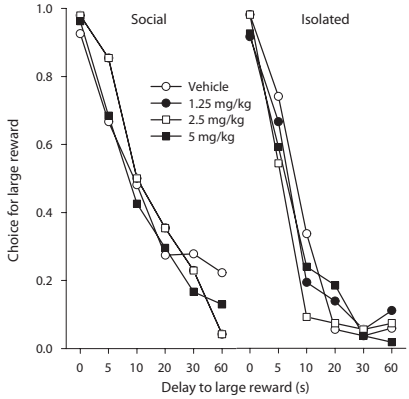

Figure 4A Effects of maprotiline in socially and isolation reared animals in the delayed reward task.

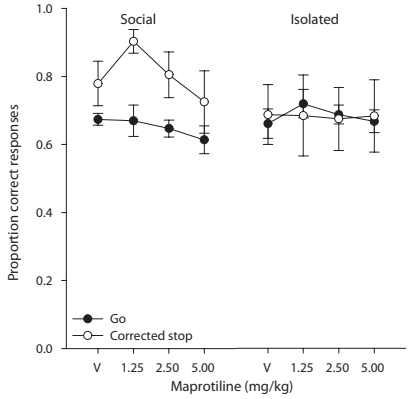

Figure 4B Effects of maprotiline in socially and isolation reared animals in the stop-signal task.

Discussion

In this report, we uncover various differences between delay aversion and response inhibition deficits. First, we showed that isolation-reared animals display both types of impulsivity compared to socially reared controls, although not throughout their entire life. Response inhibition deficits in isolation-reared animals compared to socially reared animals are present from the first time the stop-signal task can be used to measure these differences. Delay aversion is observed in the isolated animals only after six months. Second, we showed that response inhibition as a trait is much more stable than delay aversion. Third, we showed that D-amphetamine has a beneficial effect on delay aversion, but a detrimental effect on response inhibition. Finally, we showed that fluvoxamine and maprotiline had no or very small effects on both impulsivity subtypes.

Isolation-reared rats displayed a substantive response inhibition deficit compared to socially reared animals. This deficit is evidenced both by a lower stop-trial success rate and a slower stop-signal reaction time. Go-trial responding was unimpaired. Because training for the stop-signal task took three months, we could not study the onset of the response inhibition deficit. Previous research using isolated animals in the five-choice serial reaction time task show a moderate increase in response inhibition in isolated animals (Dalley et al. 2002), or no effects at all in a go/no-go task (Hellemans et al. 2005). These discrepancies may stem from the different tasks used in each of the reported studies. While the five-choice serial reaction time task is often reported to measure response inhibition (Robbins 2002), it is unclear whether this is the same type of response inhibition measured in the human stop-signal task. This is also true for the go/no-go task. While the same can be said of the animal analogue of the human stop-signal task used in the present research, procedural similarities between the human and the rat version suggest the animal stop-signal task is more likely to mimic human behavioral inhibition. Delay aversion was similar for isolated and socially reared animals for the first six months of their lives. After those six months, isolation-reared animals consistently displayed a smaller area under the preference curve, indicating a lower preference for the large reward compared to socially reared animals. Previous research demonstrated that isolation-reared animals preferred the large reward more often compared to socially reared rats during training (Hellemans et al. 2005). In that report, no long-term preferences were reported. We conclude that delayed reward tasks are acquired and executed differently by isolation-reared rats, but these differences are small and may become apparent only after several months of training.

Comparing the life span development of both impulsivity subtypes (Figure 1A and 1B), it becomes clear that response inhibition as measured by the stop-signal task is a more stable trait than delay aversion as measured by a delayed reward task. In humans, response inhibition is found to increase with age (Carver et al. 2001), with a notable slowing in older adults (Bedard et al. 2002). The minimal age studied in the present study was well over sexual maturity (around 6 weeks, and our first measurement was at around 3 months), and response inhibition deficits during ages corresponding to human childhood were not investigated. Likewise, the

experiment lasted approximately one year, and was stopped before rats reached true old age (rats can live well over a single year in captivity). Choice for the large reward summarized using the area under the preference curve clearly developed across the studied age range, with a sharp decrease in the first two months and a slower less stable decrease over the rest of the life span. In humans, no changes in delay aversion are found across the life span, both in our lab (Chapter 4), as well as in literature (Green et al. 1996), suggesting our results may be due to a training effect. Perhaps the increase in delay aversion is the result of the first phases of training, when the delay is very short. In these phases, animals learn to select the large reward. Possibly, this response pattern may persist for several months, until a more stable, lower baseline is reached.

In addition to these baseline differences, the effects of several drugs on both impulsivity subtypes were investigated. In line with clinical practice (Elia et al. 1991; Solanto 1998) and preclinical research (Cardinal et al. 2000; van Gaalen et al. 2005), D-amphetamine was effective in reducing delay aversion. The effect of D-amphetamine was larger in isolation-reared rats than in socially reared controls, a difference often reported in literature (Ahmed et al. 1995; Lapiz et al. 2003; Sahakian et al. 1975; Weiss et al. 2001; Wongwitdecha and Marsden 1995). Microdialysis studies show that administration of D-amphetamine causes a greater dopamine efflux in the nucleus accumbens of isolated rats (Jones et al. 1990; Wilkinson et al. 1994), a structure involved in delay aversion (Cardinal et al. 2001). Why isolation rearing sensitizes dopamine release is unclear, and may be attributed to dopamine synthesis or reuptake, or alterations in receptor densities, such as a down-regulation of dopamine D_2-receptors (Lapiz et al. 2003). In the stop-signal task, D-amphetamine had an opposite effect on impulsivity. While go-trial accuracy was only affected at the highest dose, stop-trial success (but not speed) dose-dependently worsened. This effect of D-amphetamine on response inhibition was not unexpected considering the disinhibitory effects of D-amphetamine in several tests (e.g. Cole and Robbins 1987). Previous studies on the effects of D-amphetamine in the rat stop-signal task, however, did not report this effect (Eagle and Robbins 2003a). In human stop-signal tasks, psychostimulants often have beneficial effects (Aron et al. 2003; Scheres et al. 2003), but this is not always found for D-amphetamine (Fillmore et al. 2005). We hypothesize that the effects of D-amphetamine are the sum of increased response inhibition caused by blockade of the serotonin or noradrenalin transporter, and response disinhibition caused by blockade of the dopamine transporter. As a result, species differences in the ratios between these levels are especially important to the outcome of that sum.

If the opposite effects of D-amphetamine on delay aversion and response inhibition are the result of manipulation of separate underlying processes, it may be possible to create drugs selective for delay aversion or response inhibition. Unfortunately, it is not possible to conclude from our data whether two separate processes are manipulated by D-amphetamine. However, in the delayed reward task, the effects of D-amphetamine are larger in the isolated than in the socially reared animals (AUPC: +45% in the socially housed animals and +100% in the isolation-reared

animals). This rearing-dependent effect is not found in the adverse effects of D-amphetamine on response inhibition (Corrected stop-trial success: –35% in socially housed animals and –25% in isolation-reared animals). This discrepancy suggests that the effects of D-amphetamine are the result of different underlying processes in delay aversion and response inhibition.

Human stop-signal task performance, in particular the SSRT, may be improved by administration of desipramine (Overtoom et al. 2003). Desipramine is an antagonist of the noradrenalin (K_i=4) and serotonin transporter (K_i=61), suggesting an involvement of noradrenalin or serotonin in response inhibition. In the present study, fluvoxamine had small effects on response inhibition. These small effects suggest at least some involvement of the serotonin system in response inhibition; a relationship that may be further explored using drugs selective for specific serotonin receptors. The experiment using maprotiline was prematurely aborted because rats developed large sores at the injection sites. The data was included in the present report because little is known about the role of noradrenalin in response inhibition, and although not complete, the data clearly indicated that maprotiline had no effects in both tasks.

In addition to a possible role in response inhibition, serotonin may also be involved in delay aversion (Mobini et al. 2000; Wolff and Leander 2002), but this is not found consistently (Winstanley et al. 2003; 2004). Desipramine had no effects on delay aversion in rats (van Gaalen et al. 2005). In the present study, the effects of fluvoxamine on delay aversion reached statistical significance. This effect could not be further explored because none of the post-hoc tests reached statistical significance. The effect, however, seems to be caused be a slight elevation of choice for the large reward in the socially housed animals at the highest dose. These data again indicate a possible role for serotonin that needs to be further explored using receptor-selective drugs. Maprotiline again had no effects.

In summary, the present study uncovered several differences between delay aversion and response inhibition. As a result, this and other studies underline the importance of making a distinction between impulsivity subtypes in diagnosis and treatment of impulsivity.

Relationship of delay aversion and response inhibition to extinction learning, aggression, and sexual behavior

Filip van den Bergh
Marjolein Spronk
Leila Ferreira
Emilie Bloemarts
Lucianne Groenink
Leon Kenemans
Berend Olivier
Ronald Oosting

Thanks to
Wim van der Wal

Impulsivity is an important symptom of many psychiatric disorders, and can be divided into two subtypes: response inhibition deficits and delay aversion. In the present study, we investigated the relationship between delay aversion and response inhibition, both to each other and to locomotion, extinction of conditioned responses, sexual behavior, and aggressive behavior. To that end, we quantified the behavior of 24 rats in several tests. To measure response inhibition, rats were trained in a stop-signal task. In this operant task, rats were rewarded food if they inhibited execution of a conditioned response after presentation of an audible stop-signal. Delay aversion was measured in an operant task in which rats made a choice between a small, immediately available reward and a large reward available after a delay. The results showed that delay aversion and response inhibition were independent. Responses during extinction and various measures of aggressive behavior were positively correlated to delay aversion. The speed of go-trials in the stop-task was correlated to non-aggressive behavior. We conclude that the role of response inhibition in various behaviors is small, but delay aversion in particular contributes to several other behaviors, such as aggressive behavior and extinction.

Introduction

Some situations call for rapid responding based on little information, and people and animals are well equipped for such situations. However, if this rapid responding is applied to situations where forethought is required, behavior may become impulsive and may seriously hamper everyday life (Evenden, 1999). Recent research suggests that at least two different processes may lead to impulsive behavior (Sonuga-Barke, 2004). Delay aversion is the first process leading to impulsivity. Impulsive individuals perceive delays as especially aversive, and therefore make decisions resulting in immediate gratification, or, if delays are unavoidable, avert attention from the long-term goal to decrease the subjective delay (Ainslie, 1975; Kuntsi et al., 2001; Sagvolden et al., 1998; Solanto et al., 2001). Intolerance to delayed rewards may be the result of alterations in the frontostriatal reward networks, including the nucleus accumbens core (Cardinal et al., 2001; Sonuga-Barke, 2003). In the second theory, impulsivity is the result of a failure to inhibit ongoing or planned responses (Barkley, 1999). This process is often described as a competition between hypothetical go- and stop-signals in which the winning signal determines whether or not a response is made (the horse-race model: Logan, 1994). Frontal and medial striatal areas are implicated in inhibiting prepotent responses (Casey et al., 1997; Eagle and Robbins, 2003a; Sonuga-Barke, 2003).

The two impulsivity subtypes are core symptoms of many psychiatric disorders. In preschool children suffering from Attention-Deficit Hyperactivity Disorder (ADHD), delay aversion is especially prominent, occurring alone in 27% of the children, and in 29% together with a response inhibition deficit (Dalen et al., 2004; Sonuga-Barke et al., 2003). Further, heroin and cocaine abusers display delay aversion (Coffey et al., 2003; Kirby et al., 1999), and a similar delay aversion is seen in smokers (Ohmura et al., 2005; Reynolds et al., 2004) and alcoholics (Petry, 2001a). Delay aversion is not just associated with addiction to drugs, as it predicts pathological gambling severity as well (Alessi and Petry, 2003; Petry, 2001b), suggesting that it is not the drugs causing changes in delay tolerance, but rather the trait causing susceptibility to addiction. Furthermore, a recent study showed that delay aversion predicts the acquisition of cocaine self-administration in rats (Perry et al., 2004). A shared underlying core deficit may also explain the high incidence of comorbid ADHD and substance-abuse disorders (Kalbag and Levin, 2005; Wilson and Levin, 2005). An association has also been found between delay aversion and classroom observations of aggressive behavior (Solanto et al., 2001). However, the association between delay aversion and aggression has not yet been extensively studied.

Response inhibition also plays a central role in ADHD, occurring alone in 15% of preschool patients and together with delay aversion in another 29% (Dalen et al., 2004; Sonuga-Barke et al., 2003). The response inhibition deficit in ADHD patients is well studied across all ages, including adult ADHD (Aron et al., 2003; Bekker et al., 2005; Quay, 1997). Other disorders in which response inhibition plays an important role are Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD) (Albrecht et al., 2005; Sergeant et al., 2002).

Clearly, both delay aversion and response inhibition are central traits important to many behaviors, both normal and pathological. Before the two impulsivity subtypes were recognized as complementary, many studies were aimed at reinforcing one theory as the main or core symptom of a disorder (Barkley, 1999; Sagvolden et al., 1998). Now that delay aversion and response inhibition are perceived as independent contributors to impulsivity, studies have focused on finding differences and similarities between the two subtypes (Dalen et al., 2004; Kuntsi et al., 2001; Sonuga-Barke, 2005; Sonuga-Barke et al., 2003), and determination of the relative contributions of both impulsivity subtypes to pathological behavior (Solanto et al., 2001). A disadvantage of the groups used in those studies is their heterogeneity. The variability within children diagnosed with ADHD can be very large, in part because both impulsivity subtypes can lead to ADHD symptoms (Nigg et al., 2005).

The aim of the present study is to determine the relationship between delay aversion and response inhibition and their involvement in various other basal behaviors. To that end, we quantified the behaviors of 24 untreated rats in a number of different tests. This approach can uniquely be used to determine the overlap between the two impulsivity subtypes because the experimental group is very homogeneous compared to human samples. Further, near-absolute control can be executed over the environment of the animals. Therefore, any associations are likely to be the result of actual overlap between the two impulsivity constructs. Finally, the influence of the two impulsivity subtypes on a number of other basal behaviors important for animal survival can reliably be measured.

Methods

Experimental design

A group of 24 rats was followed over a year and tested in many different tests for impulsivity and other possibly related constructs. The order of the different tests was the same for all animals and is described below. The order in which animals were tested on a day was varied for each test to avoid effects of time-of-day. Measurements used for this study were always at least one week apart. All operant procedures are available for download at the Medstate Notation Repository (www.mednr.com).

Animals and housing

Twenty-four male Wistar rats (HsdCpb:WU) obtained from Harlan (The Netherlands) weighing 125 g on arrival, were housed a in light (lights on from 7:00 to 19:00), temperature (21 ± 2°C), and humidity (50 ± 10 %) controlled animal facility. At the start of the study, they were housed in groups of four male rats. At this stage, animals received 15 g of standard laboratory chow per day and had free access to water. This food restriction served as an incentive for responding in the operant tasks (the stop-signal task, the delayed reward task and the extinction test). After approximately 8 months, the groups of four male rats were split and each rat

was housed in a cage together with a female companion. At this point, animals received free food and water. One animal died of an unknown cause during training for the stop-signal task procedure (within 1 month after arrival). The ethical committee on animal experiments of the Faculties of Pharmaceutical Sciences, Chemistry and Biology of Utrecht University approved the experiments.

Apparatus

The stop-signal task and the extinction test were conducted in a set of 8 Med Associates operant chambers controlled by MED-PC IV software (MED Associates, Vermont, USA). The operant chambers were equipped with a lever and a signal lamp on each side of the food magazine. An additional signal lamp was mounted above the food magazine. Tones were presented using a speaker mounted near the ceiling of the box. The delayed reward procedure was conducted in a different set of 8 Med Associates chambers equipped with a curved five-hole nosepoke wall, but lacking levers. The open field consisted of a gray PVC box (l × w × h: 75 cm × 75 cm × 40 cm) with a camera mounted in the ceiling of the test room. Up to four rats could be tested at the same time. The observation boxes used in the sexual and aggressive behavior tests were rectangular, gray PVC boxes with one transparent side (l × w × h: 60 cm × 30 cm × 40 cm) and sawdust bedding on the floor. For the aggressive behavior tests, a transparent, perforated division was used to divide the box in two compartments, the largest consisting of approximately three-quarters of the box.

Stop-signal task

The stop-signal task was adapted from Eagle and Robbins (2003b). Animals were placed in the operant chambers for 60 minutes or until they had completed 200 trials. Sessions were divided into blocks, which consisted of several successful go-trials (1 to 3, determined randomly), and a concluding stop-trial. Lever extensions and food rewards were signaled by the illumination of a light above the lever or feeder tray. At the start of a go-trial, the left lever was extended for a maximum of 60 s. A response on the left lever resulted in the retraction of that lever and extension of the right lever for a limited amount of time (the limited hold period), during which the animal had to make a response to receive a food reward. If the animal failed to respond within the limited hold period, an omission was scored, and the animal received a timeout. Stop-trials were similar to go-trials, except for a 400 ms tone that was presented immediately, or 800, 700, 600, or 500 ms before the expected response on the second lever (based on previous sessions for each animal individually, as described in the next paragraph). On stop-trials, animals had to inhibit their response on the second lever for the entire limited hold period to receive a food reward. Failure to do so resulted in a timeout. During timeouts, the houselight was extinguished for 5 s. After the timeout period, the inter-trial interval commenced automatically. The duration of the limited hold period was determined for rats individually, and was based on their previous performance. The limited hold period was defined as the maximum mean time

between pressing the left lever and the right lever plus 150 to 300 ms, and ranged between 850 ms and 1500 ms. Three measures were derived from the stop-signal task. First is the mean go response time (mRT). Second is the stop-signal response time (SSRT), calculated according to Logan (1994). The SSRT was defined as the mean of the individual SSRTs calculated for each stop-signal interval. The final measure is the corrected inhibition ratio as described in Tannock (1989). Training for the stop-signal task took approximately four months.

Delayed reward task

The delayed reward task was adapted from Cardinal et al. (2000). In a session consisting of 6 blocks of 8 trials, rats had a choice between a nosepoke hole that, if the rat poked in the hole using its nose, delivered a single food reward instantaneously, and a second nosepoke hole that delivered four food rewards, but after a delay. In the first block, this delay was 0s, but in each successive block the delay was increased until it was 60s in the final block (0s, 5s, 10s, 20s, 30s, 60s). To make sure that the rats had actually sampled both levers, the first two trials of each block were forced trials in which only one of the levers was present. Both levers were presented once in the forced trials, and the order of presentation was determined randomly. The remaining 6 choice trials were used to calculate a preference ratio for each delay. From these six ratios, the preference curve was drawn, and the area under the preference curve (AUPC, which varies from 0 to 60) was calculated. The AUPC is a theory-neutral measure for inhibition in the delayed reward task (Myerson et al., 2001). Training for the delayed reward task took approximately 2 months.

Open field test

Animals were placed in the open field for 15 minutes. Noldus Ethovision (Noldus et al., 2001) was used to track the animals and calculate the distance moved. The open field was repeated twice and the average was taken as an index of locomotion.

Extinction

After the open field test, animals were retrained on a continuous reinforcement schedule for two days, which all animals learned readily. On day 3, none of the levers resulted in the delivery of food rewards, and the number of responses made in a 30 min session was registered.

Sexual behavior

The sexual behavior procedure was adapted from Pattij et al. (2005). After the extinction test, the male animals were housed apart from each other and a companion female was introduced (see Animals and Housing section). Twice a week for two weeks, the male rats were put in an observation cage for 1 h. In the second half of that hour, a naive female rat was introduced. To induce receptivity, the females were pretreated with 50 µg estradiol 36 h in advance. Mounts, intromissions and ejaculations of the male rat were observed using Noldus Observer (Noldus et al., 2000). The first three sessions were considered training sessions, and only the data of the fourth test were used for this study.

Aggressive behavior

Animals were tested for aggression twice a week for two weeks. Male rats together with their female companions were put in an observation cage 24 h before testing. At the start of the test, the female was removed from the observation cage, and an intruder rat (weighing ~100g less than the resident) was placed inside a shielded compartment in the observation box (see Apparatus section). After 10 minutes, the division was removed, and all behavior was scored manually using Noldus Observer (Noldus et al., 2000). The following behaviors were scored from the perspective of the resident: bites, fight sequences, ano-genital sniffs, grooming by the resident of the intruder, and mounts. The last three behaviors were considered non-aggressive. Afterwards, intruders were sacrificed, shaved, and the total length of all wounds added was measured using a marking gauge (expressed in mm). Again, only the data of the fourth test were used.

Testosterone

Blood plasma testosterone was determined using an MP Biomedicals Inc. (Orangeburg, NY, USA) ImmuChemTM Double Antibody ^{125}I RIA kit. Measurements were the average of three assays determined using an optimal standard curve.

Statistics

All variables were inspected for normality and then correlated to measures of inhibitory control and delay valuation using Pearson' product moment correlation coefficient. For correlations to be considered, they had to be significant (with the level of significance set at 5%, one-tailed) as well as larger than 0.45, resulting in an explained variance (r^2) of at least 20%.

Results

As is clear from Figure 1 neither the speed of the stop-process nor the speed of the go-process is related to delay aversion. Neither the SSRT nor the mRT were correlated with the AUPC (r=0.06, NS and r=0.08, NS respectively). Similar findings are reported in humans (Dalen et al., 2004; Sonuga-Barke et al., 2003), although in some reports go response time is mildly correlated to inhibition in a delayed reward task (Solanto et al., 2001).

The correlations between the measures of impulsivity and the remaining measures are listed in Table 1. The speeds of the go- and the stop-processes are correlated (r=0.44, p=0.019). Although this correlation did not reach our criterion (r≥0.45), it may indicate that both processes are affected by a third, underlying factor. Like the stop-signal task in human subjects, the SSRT is related to the corrected inhibition rate (r=-0.64, p=0.001), with slower inhibition response times resulting in lower inhibition success (Solanto et al., 2001).

The distance moved in the open field test was not associated with any of the measures in the stop-signal task, but was mildly correlated to the behavior in the delay aversion task (r=-0.37, p=0.05). Although significant, we think this effect is too small to be of importance. The number of lever presses during the extinction session was also not correlated to any of the stop-signal task measures, but the correlation with delayed reward task performance was quite strong (r=-0.63, p=0.001).

None of the three sexual behavior parameters (mounts, intromissions and ejaculations) were related to any of the impulsivity measures. While a small correlation exists between the latency to the first mount and the AUPC (r=-0.38, p=0.05), this effect did not meet our criterion and is thus regarded as behaviorally not relevant. Further exploring the relationship between sexual behavior and impulsivity did not yield any effects (latency to the first occurrence of each of the three sexual behaviors and the number of intromissions per ejaculation). Although the correlations between delay aversion and both sexual behavior and locomotion did not reach criterion, it did raise the question whether sexual behavior and locomotor activity are correlated. Our results show that the number of mounts was not correlated to the distance moved in the open field (r=0.16, NS), however, the number of ejaculations was (r=-0.46, p=0.01). In other words, hyperactive animals have fewer ejaculations in a 30 min sexual behavior test.

A relationship between impulsivity and aggression was apparent in many of the different indexes for aggression. All non-aggressive social behavior in the test for aggressive behavior correlated with the go response time (mRT) of the stop-signal task (sum: r=0.55, p=0.004; ano-genital sniffs: r=0.58, p=0.002; grooming: r=-0.43, p=0.02; mounts: r=0.48, p=0.01). Thus, animals that made slower go-responses in the stop-signal task display more ano-genital sniffs and mounts, but less grooming behavior towards the other male in the test for aggressive behavior. In contrast, all aggressive behaviors in this test correlated negatively with the AUPC (bites: r=-0.60, p=0.002; fights: r=-0.52, p=0.006; wounds: r=-0.50, p=0.009). This means that animals that display delay aversion are also aggressive in the resident-intruder test. The number of non-aggressive behaviors was not related to the number of aggressive acts.

Testosterone levels in the blood were not associated to either impulsivity subtypes. In addition, basal plasma testosterone was not associated with sexual performance or basal aggression (see also Smith et al., 1992).

Measure	Mean ± SEM	Go response time	Stop response time	Inhibition in delayed reward task
Stop-signal task				
Go response time (mRT; s)	0.74 ± 0.04	-	0.44#	0.08
Stop response time (SSRT; s)	0.38 ± 0.03	0.44#	-	0.06
Corrected inhibition rate (%)	63 ± 5	-0.23	-0.64*	0.15
Delayed reward task				
Inhibition (AUPC)	40.3 ± 2.6	0.08	0.06	-
Open field/extinction				
Distance moved (m)	48.1 ± 1.8	-0.30	0.00	-0.21
Extinction (responses)	92.4 ± 8.6	-0.12	-0.01	-0.63*
Sexual behavior				
Number of mounts	23.0 ± 4.9	-0.22	0.11	0.38#
Number of intromissions	14.5 ± 1.3	0.32	0.02	-0.02
Number of ejaculations	2.9 ± 1.4	0.31	-0.02	0.09
Aggressive behavior				
Number of bites	6.4 ± 1.4	0.05	0.04	-0.60*
Number of fights	4.2 ± 0.8	0.02	-0.15	-0.52*
Total wounds (mm)	22.2 ± 4.6	-0.09	0.04	-0.50*
Non-aggressive behaviors	20.0 ± 2.2	0.55*	0.07	-0.03
Testosterone				
Plasma (ng/ml)	1.13 ± 0.12	-0.16	-0.30	-0.07

Table 1 Correlations between two measures of the stop-signal task (the go response time mRT and the stop response time SSRT), the delayed reward task (the area under the preference curve AUPC), and various other behaviors. * indicates a significant correlation, # indicates a correlation that reached statistical significance but did not meet the absolute criterion (r^2>20%, see Statistics section).

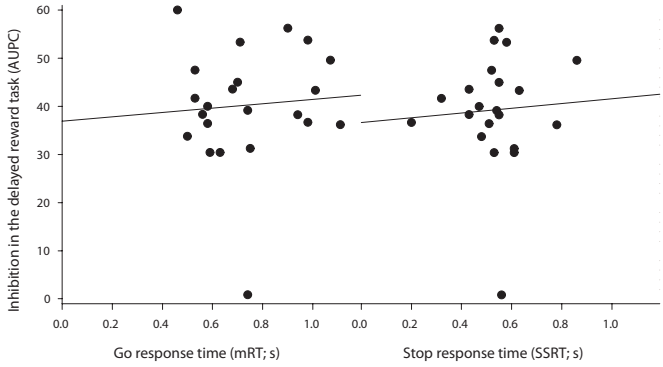

Figure 1 Correlations between two measures of the stop-signal task (the go response time mRT and the stop response time SSRT), the delayed reward task (the area under the preference curve AUPC). The correlations were not significant (left: r=0.08; right: r=0.06).

Discussion

The present article shows that response inhibition is unrelated to delay aversion in a homogeneous group of rats. In children, a similar lack of association has been reported in several studies. Dalen and colleagues (2004) found a lack of association in 3-year old children tested on a delay aversion task (in which subjects could choose between one sweet delivered after 1 s or two sweets delivered after 17 s) and a go-no go inhibition task. Sonuga-Barke (2003) and colleagues have shown that response inhibition deficits are unrelated to delay aversion in 3 to 5.5 year old children. Solanto and colleagues (2001) obtained similar results using the delayed reward task and the stop-signal task in 7 to 9.9 year-old children. The present study, using correlational research in animals also supports the conclusion that impulsivity is not a unitary construct. One of the added values of the present study lies in the homogeneity of the experimental group. All rats were members of the same strain, and had received similar treatment throughout their life. As a result, the correlations in the present study are less disturbed by unknown factors, allowing for a more accurate estimation of the overlap of the separate impulsivity constructs. Winstanley and co-workers (2004) reported that delay aversion is uncorrelated to premature responding in the five-choice serial reaction time task, an animal analogue to the continuous performance task in humans (Carli et al., 1983). The exact type of impulsivity measured in this task is still under scrutiny, however, and the measure is often referred to as "aspects of response inhibitory control" (Robbins, 2002).

In human life, impulsivity may play a detrimental role. We investigated the role of the two impulsivity types in several behaviors important to everyday life (of a rat): locomotion, learning, aggression, and sexual behavior.

Spontaneous locomotor activity was not associated with either of the impulsivity subtypes.

In children with ADHD, however, Sagvolden and colleagues (1998) found that hyperactivity may be the result of delay aversion. Children with ADHD developed hyperactivity in situations where the reward was delayed or not delivered at all (such as under extinction). Because no rewards are withheld and no such pressure is present in the open field, general locomotor hyperactivity is not related to delay aversion (as demonstrated in the present data), although specific situations such as that described above may still induce hyperactivity. This is further illustrated by the extinction sessions of the present experiment. During extinction training a reward was withheld, and an association was found in the current dataset between extinction responding and tolerance to delayed rewards. Animals that displayed a preference for immediate gratification in the delayed reward task also displayed persistent lever-pressing during the extinction session. Persistence during extinction is also one of the clearest defects in the SHR strain (Johansen and Sagvolden, 2004; 2005; Van den Bergh et al., 2005), a strain often used as a model for ADHD (Sagvolden, 2000; Sagvolden et al., 1993). Previous research in our lab has shown that extinction is also highly (up to 0.80) correlated to burst responses in the differential reinforcement of low rate responding (DRL)-72 s task (Van den Bergh et al., 2005; see Chapter 1). Because no direct comparisons between the DRL and the delayed reward task exist, any association is speculative. However, in addition to their persistence during extinction, SHR also show an increased number of burst responses in the DRL (Van den Bergh et al., 2005; see Chapter 1).

In spite of the importance of dopamine and the reward circuitry in the motivational aspects of sexual behavior (Damsma et al., 1992; Gainetdinov and Caron, 2000; Paredes and Agmo, 2004) and the involvement of dopamine and those same brain areas in delay aversion (Cardinal et al., 2001; Cardinal et al., 2000; van Gaalen et al., 2005), sexual dysfunctions such as premature ejaculation, low sex drive, or hypersexual activity are not related to ADHD or other impulsivity disorders. This independence is also reflected in the current data, as none of the correlations between impulsivity measures and parameters of the sexual behavior test reached our criterion. Some reports do exist that ADHD patients have more sexual impulsive disorders, including paraphilias (Kafka and Prentky, 1998), but such disorders are not reflected in the used animal models.

In the aggressive behavior test, a male was introduced into the territory of the resident. Animals that responded slowly in go-trials of the stop-signal task displayed more ano-genital sniffs and mounts, but less grooming behavior directed at the intruder than faster animals did. Why the direction of the correlation between go-trial speed and grooming behavior is opposite to the direction of the correlation found between the other two non-aggressive behaviors is unclear. This difference may be the result of different styles of social behavior found in fast versus slow responding animals. While the non-aggressive behaviors were correlated to the go-trial speed, the three measures of aggression correlated to tolerance to delayed rewards. Animals that chose immediate gratification were more aggressive and injure their opponents more than other animals.

A similar but more modest association between delay aversion and aggressive behavior was also found in children (Solanto et al., 2001). The associations between delay aversion and aggressive acts in rats and children provide insight into the type of impulsivity involved in aggression. A universally accepted classification of aggression in humans does not exist, but impulsive aggression is usually listed as a factor (Barratt and Felthous, 2003; Barratt and Slaughter, 1998; Barratt et al., 1997). It is often assumed that this type of aggression is related to an inability to inhibit aggressive urges (sometimes called "hair-trigger" responses: Barratt et al., 1997). The association between aggression and delay aversion (and the lack of an association with stop-signal task performance) suggests that aggressive behavior may be the result of an inability to foresee the consequences rather than a ballistic process unchecked by inhibitory control.

The correlation study described above provides further evidence for the independence of response inhibition and delay aversion. In addition, it provides evidence that both subtypes independently contribute to other behavior. The final line of reasoning for the independence of these impulsivity subtypes comes from their pharmacological differentiation. In healthy animals, D-amphetamine has been shown to have anti-impulsive effects in the delayed reward task (Cardinal et al., 2000; van Gaalen et al., 2005), while impulsivity in the stop-signal task is unaffected (Eagle and Robbins, 2003a) or elevated (Chapter 2 and 6). Such opposite effects may not be readily apparent in patients receiving methylphenidate treatment. Methylphenidate is a dopamine reuptake inhibitor and releaser, but also inhibits the noradrenalin and the serotonin transporter (Gatley et al., 1996). Dopamine reuptake inhibition may decrease delay aversion (Cardinal et al., 2000), while noradrenalin reuptake inhibition may be the active component for the positive effects of methylphenidate in the stop-signal task in humans (Overtoom et al., 2003). As a result of the broad effects of methylphenidate, it is possible that activity at some of these transporters is beneficial, while activity at others is detrimental. Also, nonresponse to methylphenidate treatment is estimated as high as 23% (Pelham et al., 1999). Research should therefore focus on the relationship between the type of impulsivity a patient displays and the improvement of symptoms as a result of treatment using more selective drugs.

From the current study, a pattern emerges in which both delay aversion and response inhibition play important but independent roles in many different types of behavior. The results indicate that especially delay aversion is an important construct underlying many other types of behavior, including extinction and aggression, and is possibly more central to many behaviors than response inhibition. Rubia (2005) argues that the delayed reward test is such a prominent feature of animal-inspired theories of impulsivity and ADHD because this test is the only available test in rats. This report and others however, show that many more tests for impulsivity exist in rats, some of them very similar to tests used in humans (Eagle and Robbins, 2003a; b; Feola et al., 2000). The delayed reward construct, however, is so prominent, because it is so valuable. Predictive validity of the delayed reward test is high, and the test correlates very well to other measures of impulsivity. This is true not only in animals (as shown in the present study), but

Delay aversion: effects of financial situation and addictive behaviors

Filip van den Bergh
Marieke Lansbergen
Koen Böcker
Lucianne Groenink
Leon Kenemans
Berend Olivier
Ronald Oosting

Thanks to
Ruud van Kooten

Delay aversion, the intolerance to waiting periods before rewards are available, is an important characteristic of human personality involved in many different types of behavior. Of particular importance is the involvement of delay aversion in various addictive behaviors. The aim of the present study was to study factors involved in delay aversion (sex, age, income) and the relationship between delay aversion and addiction (to cigarettes, alcohol, coffee and gambling). Using an internet-based questionnaire, a sample of 156 people was studied. Age and sex had no effects on delay aversion. While actual monthly income was not related to delay aversion, low perceived wealth was associated to delay aversion. In accordance to literature, smokers displayed delay aversion. Coffee consumption was related to an indifference to reward magnitude. Neither alcohol consumption nor gambling habits were related to delay aversion. We conclude that delay aversion contributes to various addictive behaviors. Whether a relationship between addictive behavior and delay aversion is found may be caused by the intensity of the addiction.

Introduction

Impulsivity is an important aspect of several mental disorders, including attention-deficit hyperactivity disorder (ADHD) and addiction (DSM-IV, 2000). Impulsive individuals are unable to adequately consider the results of their behavior (Evenden, 1999), and often display an aversion to delayed gratification (Ainslie, 1975). Immediately available reinforcers have a higher subjective value compared to reinforcers available after a waiting period (Green et al., 2004; Ho et al., 1999; Holt et al., 2003; Myerson et al., 2001). To impulsive individuals, delayed rewards lose subjective value faster than to non-impulsive individuals (Solanto et al., 2001). As a result, they either choose reinforcers that are immediately available, or divert attention away from the reinforcer and the delay, making the subjective delay appear shorter. Delay discounting is not exclusive to human subjects; pigeons, rats and mice have also been demonstrated to display hyperbolic discounting curves (Evenden and Ryan, 1996; Green et al., 2004; Isles et al., 2003; Reynolds et al., 2002). In non-human species, discounting rates are usually higher, possibly varying with metabolic speed or total expected lifetime (Green et al., 2004; Tobin and Logue, 1994).

Delay aversion is found in persons suffering from various substance abuse disorders and other addictions. Heroin- and cocaine addicts show faster discounting of delayed rewards than controls (Coffey et al., 2003; Kirby et al., 1999), and discounting is even faster if, instead of money, the abused substance is used as a reward (Kirby et al., 1999). Delay aversion is also found in smokers (Bickel et al., 1999; Ohmura et al., 2005; Reynolds et al., 2004) and alcoholics (Petry, 2001). It is unclear whether delay aversion predisposes a person to addiction, or whether substances of abuse induce delay aversion, but there is some evidence to suggest delay aversion is a predisposing trait. First, delay aversion seems unrelated to the type of substance abused, and is even found in pathological gamblers (Alessi and Petry, 2003). Second, a large number of ADHD patients display faster discounting of delayed rewards (Solanto et al., 2001), and they are at an increased risk to develop substance abuse disorders as adults (Wilens, 1998). Dutch research shows that approximately 1 in 5 addicts also suffers from ADHD (Trimbos Instituut, 2004). Finally, delay aversion predicts acquisition of cocaine self-administration in naive rats (Perry et al., 2004), also suggesting it is the trait causing the addiction. The relationship between delay aversion and addiction is further underlined by their shared underlying neural substrate. Reward areas, such as the nucleus accumbens, have been shown to also be important both in addiction and delaying gratification (Cardinal and Everitt, 2004; Cardinal et al., 2001; Cardinal et al., 2004; Di Ciano and Everitt, 2004; Gao et al., 2003). The aim of the present study was to find relationships between delay aversion as a trait and various addictive behaviors in a large, heterogeneous population. We emphasized quantity rather then careful sample selection, and therefore we delivered an internet-based questionnaire to a large group of people. The questionnaire was composed of two parts. In the first, subjects were asked about their age, sex, income, consumption of various addictive substances, including cigarettes, coffee, and alcohol,

and whether or not they enjoyed gambling. In the second part of the questionnaire, subjects were presented with a number of questions measuring their delay aversion. Subjects chose between two response alternatives, one small imaginary monetary reward available immediately, and a large reward available after a delay.

Methods

Participants

To deliver the questionnaires to the subjects, we used a viral e-mail. In this e-mail, subjects were requested to answer the questions, and to forward the e-mail message to their contacts, but only if they personally knew the experimenter. In approximately six weeks, approximately 175 subjects had responded. We then proceeded to make a selection of these subjects to create a heterogeneous, approximately normally distributed sample on a number of variables. We included 156 participants based on the following criteria: (1) subjects had to have completed the entire questionnaire, and (2) subjects had to be born between 1945 and 1985.

Procedure and questionnaire

The first part of the questionnaire consisted of the following questions (translated from Dutch): [1] Are you a man or a woman (Man/Woman), [2] When were you born? [3] What is the highest level of education you completed? [4] Please describe your financial situation (4-point scale: I'm poor/I manage/I have enough/I have more than enough) [5] How much is your monthly income? [6] Do you smoke? (Yes, less than 5 cigarettes per day/Yes, between 5 and 10 cigarettes per day/Yes, more than 10 cigarettes per day/No, I quit/No) [7] How many cigarettes did you smoke today? [8] On how many days in the week do you drink alcoholic beverages? [9] How many glasses of alcohol do you drink per week? [10] How many glasses of alcohol did you drink today? [11] On how many days in the week do you drink coffee? [12] How many cups of coffee do you drink per week? [13] How many cups of coffee did you drink today? [14] Do you gamble? (Yes/Sometimes/No).

The second part of the questionnaire was the actual delayed reward task. In this part, subjects chose between EUR 60, 90 or 120 delivered immediately, or EUR 150 delivered after a delay. The delays were 0.5, 1, 2, 3, 6, 12, 18 and 24 months. The total number of questions in the second part of the questionnaire was 24. The rewards were hypothetical (Johnson and Bickel, 2002).

Statistics

As advised by Myerson et al. (2001), we applied a theory-neutral analysis to the delay-discounting data (see also Appendix B). The three delays (for each amount) where participants shifted preference from the delayed to the immediate reward were entered into a repeated measures analysis as within-subjects variables. Per analysis, participants were classified according to answers on the first part of the questionnaire (for example, smoking habits) and entered into the

Measure	Frequency or mean ± SEM
Sample characteristics	
Sex	Men: 65
	Women: 91
Age	Men: 40 ± 1.8
	Women: 38 ± 1.3
Education (Dutch system)	Up to lower vocational: 3
	Intermediate vocational: 4
	Higher vocational: 9
	Higher vocational +: 56
	University: 84
Actual monthly income	EUR 1985 ± 143
Perceived wealth	I am poor: 18
	I manage: 52
	I have enough: 66
	I have more than enough: 20
Addictive behaviours	
Smoking	Smokes: 24
	Quit smoking: 33
	Never smoked: 99
Alcohol consumption (days/week)	mean 2.9 ± 0.18 (17 never drink alcohol)
Alcohol consumption (glasses/week)	mean 7.6 ± 0.6 (17 never drink alcohol)
Coffee consumption (days/week)	mean 5.3 ± 0.24 (24 participants never drink coffee, 93 participants drink coffee every day)
Coffee consumption (cups/week)	mean 3.3 ± 0.22
	0-1 cups: 42, 2-3 cups: 46,
	4-5 cups: 40, 6+ cups: 28.
Gambling	Gambles: 56
	Never gambles: 100

Table 1 Sample description. Values are frequencies or means ± standard errors.

analysis as separate groups. The created groups contained at least 18 participants. If the factor was continuous, it was entered into the analysis as covariate. Possible violations of sphericity demands were corrected using Huyn-Feldt's method.

In the figures, the delay is plotted at which each of the three small, immediate rewards are of equal subjective value to the large, delayed reward.

Results

Sample characteristics

In total, 175 people had completed the questionnaire. After applying the exclusion criteria, 156 participants were left. Table 1 lists the sex, age, actual monthly income, perceived financial situation, nicotine- and alcohol use, and gambling habits. The histogram for age reveals a bimodal distribution, with peaks around 50 years and 28 years. The sample was educated well over the Dutch average, with a majority of people either in or having completed university. The distribution of the rest of the variables was normal, unless otherwise specified in the various results sections.

Effect of sex, age and financial situation

The effects of sex and age are depicted in Figure 1 and 2, respectively. Men and women display similar delay aversion (F[1, 154]=2.1, NS). Figure 2 shows the effects of age using classified variables, but the analysis was conducted using participants' ages as covariates. The effect age

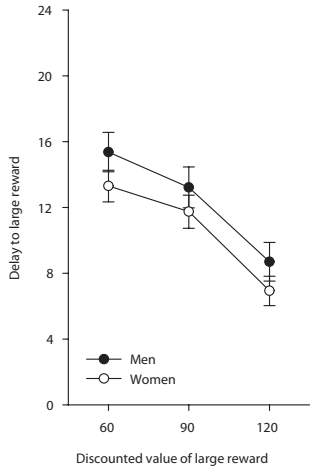

Figure 1 Men and women display similar discounting of delayed rewards. Values are means ± standard errors.

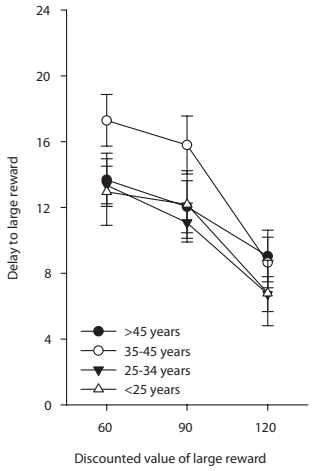

Figure 2 Effects of age on discounting of delayed rewards. Values are means ± standard errors.

has on delay aversion did not reach statistical significance (F[1, 154]=3.8, p=0.054). Although the results suggest the main effect to be obscured by an interaction between the value of the small reward and age, this interaction was also not significant (F[2, 308]=1.9, NS).

The correlation between actual monthly income and perceived financial situation (a 4-point scale) was significant, but low (r=0.392, p<0.001). Perceived wealth predicted delay aversion (F[1,154]=4.2, p=0.007; see Figure 3), but actual monthly income did not (F[1,154]=0.6, NS). This is probably due to fact that the actual income was not corrected for monthly expenses, and may therefore not be a very accurate reflection of one's financial situation. Because of the relationship between perceived wealth and delay aversion, all discussed factors are checked for a possible co-variation with perceived wealth.

Age and perceived financial situation were mildly correlated (r=-0.291, p<0.001), and correcting the repeated measures analysis for this correlation by including perceived financial situation as a covariate ablated all effects of age on delay aversion (F[1,153]=1.1, NS). In other words, any age effects on delay aversion were caused by the perceived wealth of this age group.

Smoking

Figure 4 depicts the relationship between smoking and delay aversion. Nonsmokers prefer the EUR 60 reward if the delay to the EUR 150 reward is delayed by 16 months, while smokers choose the small reward if the large reward is delayed 12 months. The difference between smokers and nonsmokers disappears when the small reward increases, as reflected in a significant interaction effect (F[6.1, 238.6]=2.4, p=0.025). Interestingly, as can be seen from the graph, participants that have quit smoking resemble smokers more than nonsmokers. Smoking habits were not associated to perceived wealth (Kendall's τ=0.07, NS).

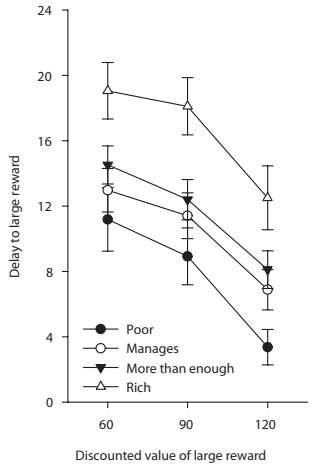

Figure 3 Effects of perceived wealth on discounting of delayed rewards. Values are means ± standard errors.

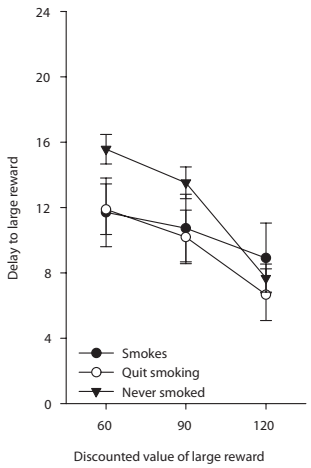

Figure 4 Effects of smoking status on discounting of delayed rewards. Values are means ± standard errors.

Alcohol consumption

The average number of days on which participants consume alcoholic beverages was not associated with delay aversion (F[11,143]=0.7, NS). The number of units consumed per week was also unrelated to delay aversion (F[27,128]=0.9, NS).

Coffee consumption

Figure 5 shows the relationship between the number of coffee consumptions per day and delay aversion. For the graph, the number of coffee consumptions was stratified for clarity, but for the analysis, the number of units was entered as a covariate (the variable was distributed normally). The number of consumptions per day interacted significantly with the delay to the large reward (F[1.5, 237]=6.0, p=0.006). As can be seen from the graph, the more coffee participants drink per day, the less sensitive the participant is to the amount of the reward. A similar interaction is found between the number of days on which participants consume coffee and delay aversion (F[12.8, 234.4]=2.1, p=0.019). Coffee consumption was not related to perceived wealth (r=0.032, NS).

Gambling

Gambling was not associated to delay aversion (F[1.4, 229.3]=1.0, NS).

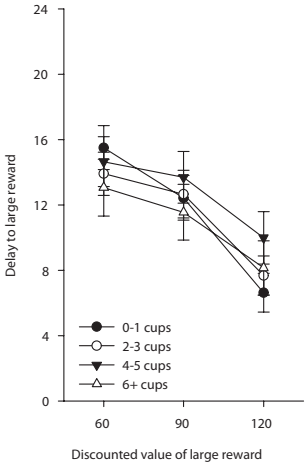

Figure 5 Effects of coffee consumption on discounting of delayed rewards. Values are means ± standard errors.

Discussion

The present study was conducted to find relationships between addictive behavior and delay aversion. Smokers displayed increased delay aversion, while coffee drinking was associated with an indifference to the size of the small reward. Both alcohol consumption and gambling were not associated to delay aversion. The strengths of the present experiment are also its limitations. We chose to maximize the number of participants rather then to carefully select homogeneous subgroups. The resulting sample was distributed normally on a number of variables, but was skewed in favor of highly educated participants. While education may affect delay discounting (Jaroni et al., 2004), there is at present no reason to believe mechanisms of addiction are different in highly educated people. Unlike many other studies, the present sample was not composed of only students.

In the current dataset, men discounted delayed rewards as fast as women. In literature, no basal differences between men and women are reported. Interestingly, one study found differences between women with and without a parental history of alcoholism, an association not found in men (Petry et al., 2002). Age was not related to delay aversion in the current dataset, contrary to previous reports of Green et al. (1996). In that report, they conclude that delay aversion decreases steeply between the ages of 20 and 30 years. In addition, they conclude that age and income interact on delay discounting rates. Our approach is not suited to uncovering such relationships, as age and income tend to vary together in a normal population (and in our sample), and strict sample selection is necessary to create sufficiently large groups. Unfortunately, Green et al. rely on the assumption that the students included in their study were from an upper income cohort. They make this assumption because the students are enrolled in an expensive private university (Green et al., 1996). The present data, however, demonstrated that income is not as important to delay aversion as participants' perception of their wealth. In fact, any effect of age on delay aversion disappeared when corrected for perceived financial situation, while actual monthly income had no such effects.

The present study confirms the relationship between smoking status and delay aversion (Bickel et al., 1999; Ohmura et al., 2005; Reynolds et al., 2004). Unlike previous reports (Bickel et al., 1999), participants that quit smoking discounted delayed rewards as fast as smokers. Our results suggest either that delay aversion predisposes people to an addiction to smoking, or that cigarettes incur lasting alterations in the brains of smokers. Bickel and colleagues included participants that had quit smoking at least one year before the study, and had smoked at least 20 cigarettes per day (1999). Our study may have included people that quit smoking very recently, or people that smoked only very briefly and were never addicted.

Our results show a significant interaction effect between coffee consumption and the magnitude of the small reward, indicating that coffee consumption is associated with an indifference to reward magnitude. In other words, high coffee consumption was associated with fast discounting of small rewards, but also with slow discounting of large rewards compared to low coffee

consumption. Many human studies on delay aversion report a magnitude effect: the observation that smaller rewards are discounted faster than larger rewards (Johnson and Bickel, 2002). However, none of these studies address the interpretation of between-subjects differences in the size of the magnitude effect. This is the first time that an association between delay aversion and coffee consumption is reported.

Of the four potentially addictive behaviors analyzed here, the present study found two to be related to delay aversion. Cigarette smoking and coffee consumption were both associated to altered discounting of delayed rewards, although not necessarily with an aversion to delayed rewards, as coffee consumption was associated with faster discounting of the small reward (EUR 60), and slower discounting of the large reward (EUR 120). Alcohol consumption and gambling were both unrelated to delay discounting. We hypothesize that the intensity of the addiction may play a role. The average education level of our sample was quite high, and the participants were obviously functioning well. Functioning in Dutch society with a smoking or coffee addiction is much easier than with an alcohol or gambling addiction. Both cigarette smoking and coffee consumption are accepted addictions in the Netherlands. While the number of non-smokers is rising, 27.9% of the Dutch population over 15 years old smokes, and 31% quit smoking (STIVORO, 2004). The current sample is not expected to contain many true alcoholics (answers in the questionnaire show that only seven people report drinking more than 21 alcoholic beverages per week). This may explain why we do not find an association to delay aversion, while an association is reported in literature (Petry, 2001). Rather, we can draw the conclusion that recreational use of alcohol is not related to delay aversion. A similar result was obtained for gambling. While some reports find that gambling is related to delay aversion (Alessi and Petry, 2003), others do not find such a relationship (Holt et al., 2003). Alessi et al. (2003) used diagnosed pathological gamblers, and Holt et al. (2003) included students that liked to gamble as assessed by a score of >4 using the 20-point SOGS scale. Since Holt's sample was composed of students, his participants obviously functioned well, and may be related to our sample more than the sample used by Alessi.

We conclude that smoking habits and consumption of coffee are related to alterations in the discounting of delayed rewards in a normal human population. Smokers seem to discount small rewards faster than nonsmokers, and ex-smokers resemble smokers more than nonsmokers. Coffee consumption is associated to faster discounting of small rewards, but slower discounting of large rewards.

Delay aversion: pharmacological links to aggression and addiction

Filip van den Bergh
Emilie Bloemarts
Lucianne Groenink
Berend Olivier
Ronald Oosting

Impulsive individuals often display an aversion to waiting for rewards. Delay aversion can be quantified in rats in a delayed reward task, in which animals choose between an immediately available, small reward, and a large reward available after a delay. Links between delay aversion and aggression, addiction and extinction of conditioned behaviour suggest a possible shared pharmacology. Therefore, we examined the effects of the dopamine D_3-receptor agonist 7-OH-DPAT, the 5-HT_{1A}-receptor agonist flesinoxan, the $5HT_{1B/1A}$-receptor agonist eltoprazine for their involvement in both addiction and aggression, and the NMDA-receptor agonist D-cycloserine, which facilitates extinction. The results show that 7-OH-DPAT slightly decreased choice for the large reward. Flesinoxan disrupted task execution by lowering choice for the large reward even at a delay of 0 s. Eltoprazine slightly increased choice for the large reward, but the 5-HT_{1B}-antagonist GR127935 had no effect. Administration of D-cycloserine also had no effect on choice behaviour. The data suggest the dopamine D_3-receptor and the 5-HT_{1B}-receptor are interesting targets for treating delay aversion impulsivity.

Introduction

Impulsivity is an important symptom of many psychiatric disorders, in particular attention-deficit hyperactivity disorder (ADHD) and mania (DSM-IV, 2000). Patients suffering from impulsivity are unable to adapt current behavior to meet future demands. Instead, their behavior is geared towards immediate action or reinforcement (Evenden, 1999). Different types of impulsivity, or pathways leading to impulsivity, are no longer seen as mutually exclusive, but rather as complementing accounts (Sonuga-Barke, 2005). In ADHD, different patients are suffering from different types of impulsivity, and sometimes several types of impulsivity are present in a single patient (Dalen et al., 2004; Sonuga-Barke et al., 2003).

One important impulsivity subtype is faster discounting of delayed rewards (Ainslie, 1975). Rewards available after a delay have a smaller reinforcing value than immediately available rewards, and this loss of value is faster in impulsive individuals (Sagvolden et al., 1998), a phenomenon called delay aversion. Delay aversion is associated to other measures of impulsivity and hyperactivity in children with ADHD (Solanto et al., 2001). Patients suffering from other impulsivity disorders, such as addiction to alcohol (Petry, 2001a), nicotine (Bickel et al., 1999; Ohmura et al., 2005; Reynolds et al., 2004), cocaine (Coffey et al., 2003), heroin (Kirby et al., 1999), and even gambling (Alessi and Petry, 2003) also display a preference for immediate gratification compared to control groups.

Several operant tests have been developed for measuring the decrease in value with delayed availability in animals. Early tests used an adjusting approach to find the indifference delay where a large reward is equal in subjective value to an immediately available small reward. In an adjusting test, the delay to the large reward is adjusted based on the animal's choices until a steady-state is reached (Ho et al., 1999; Mobini et al., 2000). However, since such dynamic approaches may in some cases measure different processes than intended (Cardinal et al., 2002), non-adjusting tasks are now often used (e.g. van Gaalen et al., 2005; Winstanley et al., 2003). In a non-adjusting approach, the session is divided into a number of blocks containing several trials. At the start of a session, the delay is set at 0 s, and the delay is increased each block regardless of the choices animals make.

Current pharmacological studies focus mainly on dopamine and serotonin. Van Galen and colleagues (2005) have conducted an in-depth survey of the involvement of dopamine in the impulsivity-lowering effects of d-amphetamine in a static procedure. Their findings indicate an important role for the dopamine D_2-receptor in the effects of d-amphetamine. A study by Evenden and Ryan (1999) shows that serotonin may play a role in the delayed reward task, as 8-OH-DPAT, a 5-HT_{1A}-receptor agonist, decreased choice for the large reward at no delay, but increased choice for the large reward at a delay of 60 s. This indifference to the delay suggests a role for serotonin in the execution of the task rather then in actual delay aversion. In the two mentioned studies, important parts of the dopaminergic and serotonergic system have been addressed, but the complexity of those two systems requires more research. The aim of the present

article is to extend the knowledge on the involvement of dopamine and serotonin in de delayed reward task. In particular, new potential drug targets are suggested by the links between delay aversion, aggressive behavior, extinction (Chapter 3), and addiction (Chapter 4). First, multiple lines of evidence suggest the D_3-receptor is involved in the reinforcement of various addictive behaviors (Heidbreder et al., 2004; Heidbreder et al., 2005), including alcohol (Thanos et al., 2005) and cocaine (Xi et al., 2005). In addition, linkage studies suggest the dopamine D_3-receptor is involved in violent behavior (Retz et al., 2003). The dopamine D_3-receptor may therefore play a role in delay aversion as well. We tested the dopamine D_3-receptor agonist 7-OH-DPAT, and we hypothesized that 7-OH-DPAT increases delay aversion. Second, positive correlations (Chapter 3) between delay aversion, aggressive behavior and addiction suggest a potential role for 5-HT_{1B}-receptor agonists, and to lesser degree 5-HT_{1A}-agonists (Olivier and van Oorschot, 2005). Therefore, agonists of the 5-HT_{1A} (flesinoxan) and 5-HT_{1B}-receptors (eltoprazine) are expected to decrease delay aversion. Finally, the positive correlation between delay aversion and the number of responses during extinction trials (Chapter 3) suggests that drugs that facilitate extinction may increase choice for the large reward. A potential target for facilitation of extinction is stimulation of NMDA receptors (Falls et al., 1992; Richardson et al., 2004), and we therefore explored the effects of D-cycloserine on delay aversion.

Methods

Subjects

Sixteen male Wistar rats (HsdCpb:WU) obtained from Harlan (The Netherlands) weighing 125g on arrival, were housed in a light (lights on from 7:00 to 19:00), temperature (21 ± 2°C), and humidity (50 ± 10 %) controlled animal facility. Animals were housed in groups of four and received 15g of standard laboratory chow per day and had free access to water. The ethical committee on animal experiments of the Faculties of Pharmaceutical Sciences, Chemistry and Biology of Utrecht University approved the experiments.

Apparatus

Sixteen operant cages (MED Associates) controlled by MED-PC IV software were used. The boxes (l × w × h: 30 cm × 24 cm × 21 cm) were equipped with a houselight and a central food magazine in which 45 mg Noyes precision pellets (formula P) were delivered. Retractable levers were located on the left and right of the food magazine, and signal lamps were located above the levers and the food magazine.

Procedure

The delayed reward task was adapted from Cardinal et al. (2000). In a session consisting of 5 blocks of 8 trials, rats had a choice between a lever that delivered a single food reward instantaneously, and a second lever that delivered four food rewards, but after a delay. In the first block, this delay is 0s, but each block the delay was increased until it is 40s in the final block (0s, 5s,

10s, 20s, 40s). Total trial length was 10 s longer than the delay used in that block. To make sure that the rats had actually sampled both levers, the first two trials of each block were forced trials in which only one of the levers was present. Both levers were presented once in the forced trials, and the order of presentation was determined randomly. The remaining 6 choice trials were used to calculate a preference ratio for each delay. The duration of a session was approximately 25 minutes. Training for the delayed reward task took approximately 2 months.

Statistics

Per experiment, two analyses were made. The first is a repeated measures ANOVA of the preference for the large reward per block, with the delay to the large reward and the dosage as within-subjects variables. Post-hoc tests of the different dosages were corrected for comparisons with vehicle only. Post-hoc test were also conducted to explore effects of several drugs on the first block of the test (0 s delay). These tests were also corrected for comparison to the vehicle only. In addition to analyzing the raw choice data, the data was also reduced to the area under the preference curve (AUPC). This area reflects a theory-neutral index of inhibition in the delayed reward task (Myerson et al., 2001), but is not sensitive to interactions between drug administration and the delay to the large reward. AUPCs were analyzed in a separate repeated measures ANOVA with dose as a within-subjects factor. Again, post-hoc tests of dose effects were corrected for the comparisons to the vehicle only. Significance levels for all tests were set at 5%. All data was visually inspected for normality, and log-transformed if the criteria were not met. Huyn-Feldt corrections were applied to the degrees of freedom if data did not meet sphericity demands.

Drugs

The following drugs and dosages (in mg/kg) were used: D-amphetamine HCl (0.25, 0.5, 1), 7-OH-DPAT HBr (0.03, 0.1, 0.3), flesinoxan HCl (0.3, 1, 3), eltoprazine HCl (0.25, 0.5, 1), GR127935 (0.3, 1, 3), D-cycloserine (3.25, 15, 30). All drugs were dissolved in saline and administered subcutaneously 2 ml/kg, 30 minutes before testing. Dosages were based on the salt weights. Animals were tested daily in Latin square designs. Between drug tests, animals had a 3-day break.

Results

D-amphetamine

D-amphetamine (Figure 1) significantly increased choice for the large reward ($F[3, 42]=5.8$; $p=0.002$). This impulsivity attenuating effect was present at 0.25 and 0.5 mg/kg ($p=0.003$ and $p=0.03$ respectively). Analysis of the AUPC (Table 1) yielded similar results ($F[3, 42]=4.4$; $p=0.009$; 0.25 mg/kg: $p=0.036$; 0.5 mg/kg: $p=0.03$).

7-OH-DPAT

The dopamine D_3-receptor agonist 7-OH-DPAT (Figure 2) had no overall effects on choice ($F[2.2, 27.2]=2.3$, NS), and an analysis of the AUPC (Table 1) yielded similar results ($F[3, 39]=1.1$, NS). Closer inspection of Figure 2 reveals that effects of the higher dosages of 7-OH-DPAT may obscure the effects of the lowest (0.03 mg/kg) dose in the analysis. To explore this effect of 7-OH-DPAT, a separate analysis was conducted comparing this dose against vehicle only. The results show that animals pretreated with 0.03 mg/kg 7-OH-DPAT selected the smaller reward more often compared to animals that received a vehicle injection ($F[1, 14]=6.2$, $p=0.026$).

Flesinoxan

The 5-HT_{1A}-receptor agonist flesinoxan increased the choice for the small reward, but only if the delays were short (reflected in a delay × dose interaction: $F[9.3, 84]=2.4$, $p=0.013$; Figure 3). To further explore this effect, the 0 s delay block was analyzed separately. The results showed that 1 and 3 mg/kg flesinoxan significantly decreased choice for the large reward, even without a delay ($F[1.4, 19.7]=8.4$, $p=0.005$; 1 mg/kg: $p=0.042$; 3 mg/kg: $p=0.012$). The AUPC (Table 1) was not sensitive to this effect of flesinoxan (F1.4, 21.1]=0.9, NS).

D-amphetamine	**mean**	**± SEM**	**7-OH-DPAT**	**mean**	**± SEM**
Vehicle	6.3	± 1.5	Vehicle	9.5	± 2.8
0.125	7.2	± 1.8	0.03*	5.7	± 1.1
0.25*	10.4	± 2.3	0.1	7.6	± 1.5
0.5*	11.7	± 2.6	0.3	8.3	± 2.1
Flesinoxan	**mean**	**± SEM**	**Eltoprazine**	**mean**	**± SEM**
Vehicle	10.3	± 2.6	Vehicle	7.5	± 1.8
0.13*	9.8	± 2.0	0.25	7.3	± 1.9
0.25	8.9	± 2.1	0.5*	9.3	± 1.8
0.5*	8.2	± 1.9	1	7.1	± 1.6
GR127935	**mean**	**± SEM**	**D-cycloserine**	**mean**	**± SEM**
Vehicle	9.0	± 2.0	Vehicle	9.1	± 2.2
0.3	9.5	± 2.3	3.25	9.1	± 2.3
1	10.2	± 2.3	15	10.1	± 2.7
3	9.3	± 2.4	30	8.1	± 2.1

Table 1 Effects of various drugs on the area under the preference curve. Values represent means ± SEM. Significant changes compared to vehicle are marked with *.

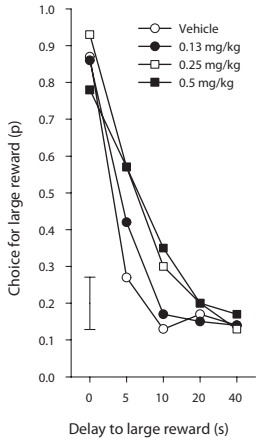

Figure 1 Effects of D-amphetamine on the preference curve. A single error bar representing twice the standard error of the mean is shown.

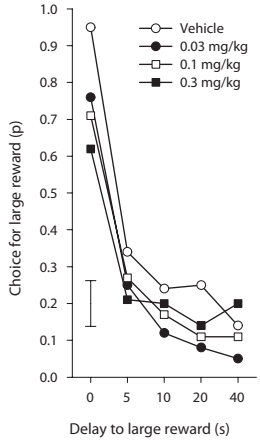

Figure 2 Effects of 7-OH-DPAT on the preference curve. A single error bar representing twice the standard error of the mean is shown.

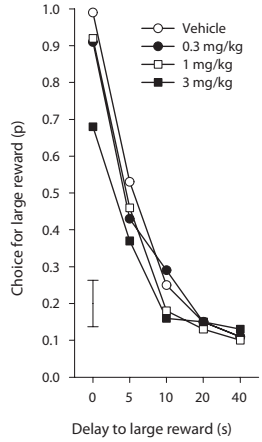

Figure 3 Effects of flesinoxan on the preference curve. A single error bar representing twice the standard error of the mean is shown.

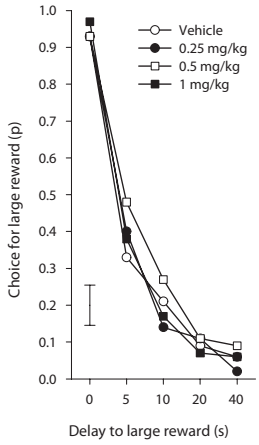

Figure 4 Effects of eltoprazine on the preference curve. A single error bar representing twice the standard error of the mean is shown.

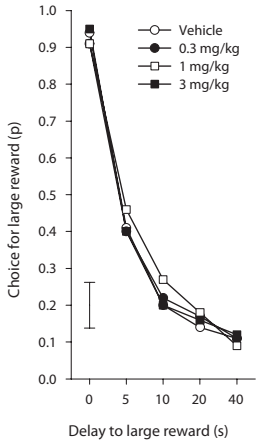

Figure 5 Effects of GR127935 on the preference curve. A single error bar representing twice the standard error of the mean is shown.

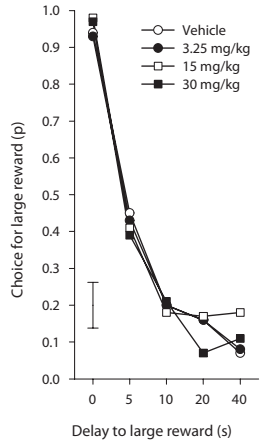

Figure 6 Effects of d-cycloserine on the preference curve. A single error bar representing twice the standard error of the mean is shown.

Eltoprazine

The 5-$HT_{1A/1B}$-receptor agonist eltoprazine (Figure 4) had no effects on choice for the large reward (F3, 42]=2.4, p=0.077). The AUPC seemed mildly more sensitive to the effects of eltoprazine, but the effects also did not reach significance (F[3, 42]=2.7, p=0.059). Closer examination of the preference curve revealed that the highest and lowest dosages of eltoprazine were not effective, but at 0.5 mg/kg, choice for the large reward was increased (F[1,14]=5.1, p=0.04).

GR127935

The effects of the 5-HT_{1B}-receptor antagonist GR127935 are shown in Figure 5. GR127936 had no effects on the preference curve (F[3, 45]=0.4, NS) or the AUPC (F[3, 45]=0.4, NS).

D-cycloserine

As seen in Figure 6, the NMDA-receptor agonist d-cycloserine had no effects on choice (F[3, 45]=0.8, NS). Analysis of the AUPC (see Table 1) led to the same conclusion (F[3,45]=1.5, NS).

Discussion

In the present study, we assessed the effects of a variety of different psychoactive drugs on delay aversion using a delayed reward task. D-amphetamine was selected as a reference drug, as psychostimulants have been demonstrated to be effective in this task before (van Gaalen et al., 2005; Chapter 2). Indeed, D-amphetamine dose-dependently increased choice for the large reward.

The dopamine D_3-receptor is an interesting drug target for the modulation of delay aversion. Addiction and delay aversion are intimately linked (Chapter 4), and the D_3-receptor plays an important role in addiction (Heidbreder et al., 2005). In the present study, administration of the dopamine D_3-receptor agonist 7-OH-DPAT decreased choice for the large reward at a low dose, and this effect disappeared at higher dosages. Interestingly, the direction of this effect is the same as for the dopamine D_1-antagonist SCH23390 (van Gaalen et al., 2005). The D_3-receptor is primarily found in the limbic system, in particular in the (shell of the) nucleus accumbens and the islands of Calleja (Bouthenet et al., 1991; Sokoloff et al., 1992b). Many (63% in the nucleus accumbens and up to 79% in other areas) neurons containing D_3-receptors also express D_1-receptors, and stimulation of the two receptors by selective agonists have been demonstrated to have opposite effects on c-fos expression (Ridray et al., 1998; Schwartz et al., 1998). This opposite role is also reflected in the similar effects of D_1-receptor antagonists and D_3-receptor agonists in delay aversion. At this moment, it is unclear why this effect of 7-OH-DPAT disappears at higher dosages, although a similar effect has been reported in the elevated plus maze (Rogoz et al., 2004). Perhaps these effects may be attributed to binding of 7-OH-DPAT to the D_2-receptor at higher dosages (Damsma et al., 1993; Sokoloff et al., 1992a)

Several lines of evidence support the link between delay aversion and addiction. First, delay

aversion in rats predicts acquisition of cocaine self-administration (Perry et al., 2004). Second, people addicted to alcohol (Mitchell et al., 2005; Petry, 2001a), nicotine (Ohmura et al., 2005; Reynolds et al., 2004), cocaine (Coffey et al., 2003), heroine (Kirby et al., 1999), and gambling (Alessi and Petry, 2003; Petry, 2001b) all display delay aversion, and discount delayed rewards faster than controls (see also Chapter 4). Blockade of dopamine D_3-receptors has been demonstrated to attenuate acquisition and expression of addictive behavior to many of the mentioned substances in various behavioral tests (Andreoli et al., 2003; Ashby et al., 2003; Gilbert et al., 2005; Thanos et al., 2005; Xi et al., 2004; Xi et al., 2005). Dopamine D_3-receptors may also be involved in violent behavior as demonstrated in a linkage study (Retz et al., 2003). We demonstrated that aggressive rats are impulsive in the delayed reward task (Chapter 3), lending further credibility to the dopamine D_3-receptor as a potential target for the treatment of pathological delay aversion.

As stated above, aggressive behavior is linked to delay aversion (Chapter 3). Although no drugs developed specifically for the treatment of aggression are commercially available, a class of serotonin agonists called serenics has anti-aggressive properties (Olivier et al., 1994; Ratey and Gordon, 1993). These drugs target 5-HT_{1B}-receptors, and to a lesser degree 5-HT_{1A}-receptors, although the latter are less specific to aggressive behavior (Olivier and van Oorschot, 2005). The 5-HT_{1B}-receptor is of particular interest for delay aversion, as stimulation of that receptor also decreases reinstatement of cocaine seeking behavior after extinction (Acosta et al., 2005). In the present article, we tested the 5-HT_{1A}-receptor agonist flesinoxan, the mixed 5-$HT_{1B/1A}$-agonist eltoprazine and the 5-HT_{1B}-receptor antagonist GR127936. Flesinoxan lowered choice for the large reward in the first block where the delay to the large reward was 0 s, but this effect disappeared at longer intervals. This effect of flesinoxan is very similar to the effects of 8-OH-DPAT, another 5-HT_{1A}-receptor agonist, on delay aversion (Evenden and Ryan, 1999). The change in the slope of the preference curve as observed after administration of flesinoxan is very different from the change of the height of the preference curve induced by 7-OH-DPAT administration, and reflects a problem in the execution of the task rather than an increase in delay aversion. In addition, behaviorally inert dosages of 8-OH-DPAT may attenuate the anti-impulsive effects of D-amphetamine in the delayed reward task (Winstanley et al., 2005), indicating that serotonin may be involved in response modulation rather than in the actual preference. Agonists of the 5-HT_{1A}-receptor have a dual effect: binding to the autoreceptor causes inhibition of serotonin release, while binding to the postsynaptic receptor mimics released serotonin (de Boer and Koolhaas, 2005; De Groote et al., 2002). Since a serotonin depletion by 5,7-DHT also attenuates the anti-impulsive effects of d-amphetamine (Winstanley et al., 2003), the effects of 5-HT_{1A}-receptor agonists may be due to binding to the autoreceptor and a resulting decrease in serotonin availability.

The 5-$HT_{1B/1A}$-receptor agonist eltoprazine slightly increased choice for the large reward and at one dose, an expected effect considering eltoprazine's effectiveness as an anti-aggressive drug

(Olivier et al., 1995; Olivier and van Oorschot, 2005). The effect was rather modest, however, and more research should be directed at the exact circumstances under which eltoprazine can lower delay aversion. Like the 5-HT_{1A}-receptor described above, the 5-HT_{1B}-receptor is also expressed pre- and post-synaptically (de Boer and Koolhaas, 2005). Multiple lines of evidence suggest that the 5-HT1B-autoreceptor is responsible for eltoprazine's efficacy in aggression (Olivier and van Oorschot, 2005), but if the mechanism of eltoprazine is the same in the delayed reward task remains speculative. Like 7-OH-DPAT, the effects of eltoprazine are strongly dependent on the used dosage. In aggression tests, De Boer et al. (1999) report a similarly shaped curve, especially for exploratory behavior.

To expand on the role of 5-HT_{1B}-receptors, we tested the antagonist GR127936. In the present study, GR127935 had no effect on choice behavior. Evenden and Ryan (1999) used several serotonin receptor antagonist (for 5-HT_{1A}, 5-HT_2, and 5-HT_3-receptors), and also report no alterations on delay aversion. They conclude that serotonin does not exert tonic control over the execution of the delayed reward task. The present data confirms that hypothesis and extends the results for the 5-HT_{1B}-receptor.

Finally, in addition to the links between delay aversion, addiction and aggressive behavior, delay aversion and extinction are also correlated (Chapter 3). In extinction tests, conditioned behavior is no longer reinforced, and the occurrence of the conditioned response is taken as an index of extinction. Several manipulations have effects on extinction of conditioned behavior, including serotonin depletion by the serotonin synthesis inhibitor PCPA (Beninger and Phillips, 1979). The links between serotonin and delay aversion are discussed extensively in the previous paragraphs. In addition to serotonin, the glutamate system is also important in extinction, as has been shown in various extinction tasks. Blockade of NMDA-receptors by local infusion of AP5 into the amygdala slows fear extinction (Falls et al., 1992). Extinction may also be facilitated by stimulation of NMDA-receptors by d-cycloserine (Ledgerwood et al., 2005; Walker et al., 2002), a drug now used in combination with exposure therapy to facilitate extinction of fear (Ressler et al., 2004; Richardson et al., 2004). In the present study, D-cycloserine had no effects on delay aversion. Perhaps the effects of D-cycloserine are specific to extinction of fear, although Port and Seybold (1998) reported effects of D-cycloserine on extinction of lever-pressing for food.

The present study investigated several drug targets inspired by the correlation between delay aversion and aggressive behavior, addiction and extinction. In addition, the data demonstrate a multitude of different effects drugs can have on choice behavior in the delayed reward task, including increased and decreased choice for the large reward, and interactions between the drugs and the delays to the large reward. Potential new drug targets identified in this study were the dopamine D_3-receptor and the 5-HT_{1B}-receptor, both new targets that have not been investigated in the context of delay aversion before.

Eltoprazine, a serotonin 1A/1B receptor agonist, resembles D-amphetamine on different measures of impulsivity

Filip van den Bergh
Koen Westphal
Emilie Bloemarts
Climmy Cruijen
André Klompmakers
Jasper Lipsch
Herman Westenberg
Lucianne Groenink
Emer Leahy
Berend Olivier
Ronald Oosting

Response inhibition deficits and delay aversion, the inability to wait for rewards, are defining characteristics of impulsivity. In rats, response inhibition can be measured in an operant task in which animals are rewarded for the inhibition of a prepotent response if an audible stop-signal is presented. Delay aversion is quantified in an operant task in which rats choose between an immediately available, small reward, and a large reward available after a delay. Delay aversion is correlated with aggressive behaviour, and we therefore examined the effect of eltoprazine, an anti-impulsive drug acting on the 5-HT_{1B}-receptor. Eltoprazine, like the reference drug D-amphetamine, decreased response inhibition and increased choice for the large reward. High dosages of D-amphetamine, unlike eltoprazine, caused breakdown of performance in both tasks. Microdialysis reveals that eltoprazine has no effects on dopamine in the striatum at behaviourally effective dosages, and we conclude that the mechanism by which eltoprazine acts on response inhibition and delay aversion is very different from D-amphetamine. This unique mechanism of eltoprazine may be useful in the treatment of impulsivity.

Introduction

Impulsivity, the inability to adequately consider the result of one's behavior, is an important characteristic of many mental disorders. For some of these disorders, impulsivity is a defining characteristic, such as in attention-deficit hyperactivity disorder (ADHD) and mania. Until recently, the impulsivity field was divided into proponents of at least two different theories on the principal deficit in impulsive patients (mainly ADHD). In the first theory, the impulsive individual is unable to inhibit the prepotent response until a proper evaluation of the situation is complete, and the response is executed (response inhibition, see Barkley, 1999; Logan, 1994). In this case, the post-hoc valuation of the response outcome may be similar to non-impulsive people, but it is too late. In the second theory, the valuation of the response outcome is skewed towards immediate gratification, even if waiting may maximize the reward (delay aversion, see Sagvolden et al., 1998). In this second theory, the impulsive individual has enough time to complete his valuation, but the impulsive choice is favored over the more rational choice. It is important to note that many situations exist where swift responses or immediate gratification are required. Impulsivity is only pathological if such behavior is also consistently applied in inappropriate situations (Evenden, 1999). Recent research suggests that the two theories of impulsivity are complementary rather then competitive, and both processes may lead to impulsive behavior, and possibly other symptoms of mental disorders as well (Dalen et al., 2004; Solanto et al., 2001; Sonuga-Barke, 2004; Sonuga-Barke et al., 2003).

Strategies for the treatment of impulsivity mostly rely on psychostimulants. Methylphenidate is the treatment of choice for ADHD, and nonresponders to methylphenidate often do respond to d-amphetamine. Although generally effective, the use of psychostimulants is associated with a number of side effects, including insomnia, anorexia, headache, and stomach problems (Bolanos et al., 2003; Goldman et al., 1998). Also, psychostimulants are potential drugs of abuse, an unwanted side effect considering the higher vulnerability of drug dependency in ADHD patients (Wilens, 1998). For the past two decades, many studies have suggested a role for serotonin (5-hydroxytryptamine, or 5-HT) in impulsivity (Soubrié, 1986), but the serotonin neuromodulatory system is complex, and the function of serotonin in impulsivity is often unclear. Fourteen different serotonin receptors exist in the rodent brain, most of them G-protein coupled, but the 5-HT_3-receptor is an ion-channel. Further, 5-HT_{1A} and 5-HT_{1B}-receptors are located both pre- and postsynaptically, with opposite functions with regard to serotonin functioning. Presynaptically, agonists binding to the 5-HT_{1A} and 5-HT_{1B}-receptors limit serotonin release (De Groote et al., 2002; Knobelman et al., 2000), while those same drugs mimic released serotonin when binding to postsynaptic receptors (Morikawa et al., 2000), located on non-serotonergic neurons. As a final complication, 5-HT_{1B}-receptors are different in humans and rodents, and are called h 5-HT_{1B} and the r 5-HT_{1B}-receptor, respectively (Hartig et al., 1996).

The complexity of the serotonin system, combined with the two subtypes of impulsivity, can

lead to complex interactions. First, a serotonergic manipulation may have different effects on these impulsivity types. For example, serotonin depletion by i.c.v. infusion of the neurotoxin 5,7-DHT can lead to decreases in response inhibition (Harrison et al., 1997), while delay aversion is unaffected (Winstanley et al., 2003), or increased (Mobini et al., 2000). Second, different serotonin receptors may have opposite functions in the same model. For example, 5-HT_{2A} and 5-HT_{2C}-receptors have opposite effects on premature responses in the five-choice serial reaction time task, a putative test for response inhibition (Winstanley et al., 2004). Third, the same drug can have opposite effects after repeated compared to acute administration. Buspirone, a partial agonist of the 5-HT_{1A}-receptor, increases delay aversion acutely, but has attenuating effects after chronic administration (Liu et al., 2004). Perhaps as a result of these complex interactions, no drugs thus far have been developed based on the serotonergic system to treat impulsivity. However, agonists of the 5-HT_{1B}-receptor such as eltoprazine, have anti-aggressive properties in animals (Olivier and van Oorschot, 2005), and aggressive behavior has clear ties to certain types of impulsivity, as reflected by correlations in humans (r=0.40; Solanto et al., 2001), and in animals (r≤0.60; see Chapter 3)

We evaluated eltoprazine as a putative anti-impulsive drug, and compared the properties of eltoprazine to d-amphetamine in tests for different types of impulsivity. The stop-signal task was used to test the ability to withhold prepotent responses (Eagle and Robbins, 2003b). To test for delay aversion, the delayed reward test was used (Cardinal et al., 2000). The most likely target for anti-impulsive drugs is the dopaminergic system (van Gaalen et al., 2005). Because the behavioral effects of eltoprazine and d-amphetamine in these two paradigms were highly comparable, we hypothesized that the behavioral effects might be due to similar effects on the dopaminergic system. Therefore, eltoprazine's effects on both dopamine and serotonin concentrations were assessed using in vivo microdialysis in the striatum, an area implicated in certain types of impulsivity (Eagle and Robbins, 2003a).

Methods

Behavioral studies

Subjects

Male Wistar rats (HsdCpb:WU) obtained from Harlan (The Netherlands) weighing 125g on arrival, were housed in a light (lights on from 7:00 to 19:00), temperature (21 ± 2°C), and humidity (50 ± 10 %) controlled animal facility. Animals were housed in groups of four and received 15 g of standard laboratory chow per day and free water. Sixteen animals were assigned to the stop-signal task, and twelve were assigned to the delayed reward task. Besides the training procedure, animals were treated the same. The ethical committee on animal experiments of the Faculties of Pharmaceutical Sciences, Chemistry and Biology of Utrecht University approved the experiments.

Apparatus

Sixteen operant chambers (MED Associates) controlled by MED-PC IV software were used. All boxes were equipped with a houselight and a central food magazine in which 45 mg Noyes precision pellets (formula P) were delivered. Eight extra tall boxes (30 cm L × 24 cm W × 29 cm H) were used for the delayed reward task and were equipped with a curved five-hole response wall opposite to the food magazine. In each of these five holes, a light stimulus could be presented, and nose poke responses could be registered. The left- and rightmost holes were not used in the delayed reward task. The eight standard height boxes (30 cm L × 24 cm W × 21 cm H) used for the stop-signal task were equipped with retractable levers to the left and right of the food magazine, and signal lamps over the levers and the food magazine.

Procedure

The stop-signal task was adapted from Eagle and Robbins (2003). Animals were placed in the skinnerbox for 60 minutes or until they had completed 200 trials. Sessions were divided into blocks, which consisted of several successful go-trials (1 to 3, determined randomly), and a concluding stop-trial. Lever extensions and food rewards were signaled by the illumination of a light above the lever or feeder tray. At the start of a go-trial, the left lever was extended for a maximum of 60 s. A response on the left lever resulted in the retraction of that lever and extension of the right lever for a limited amount of time (the limited hold period), during which the animal had to make a response to receive a food reward. If the animal failed to respond within the limited hold period, an omission was scored, and the animal received a timeout. Stop-trials were similar to go-trials, except for a 400 ms tone that was presented immediately, or 800, 700, 600, or 500 ms before the expected response on the second lever (based on previous sessions for each animal individually; see below). On stop-trials, animals had to inhibit their response on the second lever for the entire limited hold period to receive a food reward. Failure to do so resulted in a timeout. During timeouts, the houselight was extinguished for 5 s. After the timeout period, the inter-trial interval commenced automatically. The duration of the limited hold period was determined for rats individually, and was based on their previous performance. The limited hold period was defined as the mean reaction time between pressing the left lever and the right lever plus 150 to 300 ms, and ranged between 850 ms and 1500 ms. Three measures were derived from the stop-signal task. First is the mean go response time (mRT). Second is the stop-signal response time (SSRT), calculated according to Logan (1994). The SSRT was defined as the mean of the different SSRTs calculated for each stop-signal interval. The final measure is the corrected inhibition ratio as described by Tannock (1989). Training for the stop-signal task took approximately four months.

The delayed reward task was adapted from Cardinal et al. (2000). In a session consisting of 6 blocks of 8 trials, rats had a choice between a nosepoke hole that, if the rat poked in the hole using its nose, delivered a single food reward instantaneously, and a second nosepoke hole that delivered four food rewards, but after a delay. In the first block, this delay is 0s, but each block

the delay was increased until it is 60s in the final block (0s, 5s, 10s, 20s, 30s, 60s). To make sure that the rats had actually sampled both choices, the first two trials of each block were forced trials in which only one of the nosepoke holes was illuminated. Both nosepoke holes were illuminated once in the forced trials, and the order of presentation was determined randomly. The remaining 6 choice trials were used to calculate a preference ratio for each delay. Training for the delayed reward task took approximately 2 months.

Statistics

All data was visually inspected for normality, and transformed if the criteria were not met. For the stop-signal task data, the corrected inhibition percentage was calculated according to Equation 1 (Tannock et al., 1989). Mean reaction times and success in go-trials and the corrected inhibition rate were analyzed using univariate analysis of variance. Delayed reward task data was reduced to the area under the preference curve (AUPC). This area reflects a theory-neutral index of inhibition in the delayed reward task (Myerson et al., 2001). The AUPC was also entered into a repeated measures ANOVA. Post-hoc comparisons were corrected using Dunnett's approach with the vehicle as the control category. Significance levels were set at 5%.

Microdialysis studies

Subjects

Male Wistar rats (HsdCpb:WU) obtained from Harlan (The Netherlands) weighing between 350 g and 500 g at the time of testing, were housed under similar conditions as the animals described in the behavioral studies. These rats had free access to food and water, and were handled prior to the experiment. The rats were randomly assigned to the four different dosage groups.

Surgery

Animals were anaesthetized with a mixture of isoflurane, N_2O and O_2 (6% for induction, 2% for maintenance). The head was shaven and xylocaine (2%) was applied to the skin prior to making the incision. The stereotaxic instrument was equipped with a mouthpiece for anesthesia, and animals were placed on a heating pad during the surgery. Probes with an active dialysis surface of 2 mm were used (Microbiotech MAB.4.7.2.Cu). Coordinates for the microdialysis probe were AP +0.2, ML –3.0, DV –7.0, all relative to dura and bregma with the toothbar set at 3.3mm. The probes were tightened to the skull using dental cement around 3 achor screws. After surgery, rats were injected with 1ml of saline and placed individually.

$$P_i' = \frac{P_i - (P_o \times P_i)}{1 - (P_o \times P_i)}$$

Equation 1 Method used to correct the raw inhibition probability for omissions made during go-trials. P_i' is the corrected inhibition probability, P_i is the raw inhibition probability in stop trials, and P_o is probability of omissions in go-trials.

Procedure

Microdialysis experiments commenced 24 h after surgery. The probes were perfused with Ringer solution (147 mM NaCl, 2.3 mM KCl, 2.3 mM $CaCl_2$, 1.0 mM $MgCl_2$) at a flow of 0.09 ml/h using a high precision pump. Samples were collected every 30 min in vials containing 15 µl 0.1M acetic acid, and stored at -80˚C until HPLC analysis. All animals subjected to two dosages, with an additional session 24 h after the first. Each experimental day, 5 to 6 animals were tested in a balanced design. Following the second session, animals were decapitated, and the brains were extracted for later verification of the positioning of the probe. The brains were fixed in 4% formaldehyde solution for at least 2 days, and were then transferred to a 15% saccharose solution. The brains were cut into 60 µm slices, and stained with cresyl-violet. Using a microscope, probe positioning was verified, and animals with incorrectly placed probes were excluded from all analyses.

HPLC-ECD analysis

DA and DOPAC were analyzed by HPLC with electrochemical detection. Samples of 25 µl were injected onto an Inertsel ODS-3 column (3 µM, 2.1x100mm, Aurora Borealis, The Netherlands) using a Gynkotek/Dionex pump (model P580) and a Gilson (Model 231) autosampler. Detection was performed at 35˚C with an electrochemical detector (Intro ECD, Antec Leyden, Leiden, The Netherlands) set at a potential of 600 mV against an Ag/AgCl reference electrode. The signal was analyzed using Gynkotek software. The mobile phase consisted of 0.05 M acetic acid, 60 mg/l heptane sulphonic acid sodium salt, 100 mg/l EDTA, 5% methanol, at pH 4.65. Flow rate was 0.3 ml/min.

Statistics

A baseline composed of the data of the first 3 samples of 30 min was calculated and all data were calculated relative to that baseline. The average of the baseline was compared to the average of the samples from 30 to 150 min after eltoprazine administration in a repeated measures ANOVA with the drug as a between-subjects factor. Dunnett's test was used to compare the effects of the three eltoprazine dosages to vehicle administration. Correlations were always Pearson's correlations. Significance levels were set at 5%.

Drugs

For the behavioral tests, eltoprazine HCl (0.25, 0.5 and 1.0 mg/kg) and D-amphetamine HCl (0.25, 0.5 and 1.0 mg/kg) were dissolved in saline and administered subcutaneously 2 ml/kg, 30 minutes before testing. For the microdialysis studies, different dosages of eltoprazine were used (1, 3 and 10 mg/kg). The probes were connected 90 minutes before administration of eltoprazine (designated timepoint 0). All dosages were based on the salt weights.

Results

Behavioral studies

Stop-signal task

Go and stop-trial success is depicted in Figure 1. A high stop-trial success is interpreted as low impulsivity. Eltoprazine had no effect on performance in the go trials (F[3,27]=1.2, NS). Performance in stop-trials however, was significantly and dose-dependently impaired by administration of eltoprazine (F[3,27]=7.3, p=0.001. Post-hoc tests showed that performance was significantly worsened at all dosages. D-amphetamine displayed a similar profile in the stop-signal task, although go-performance was also significantly impaired (F[1.2, 14.4]=5.2, p=0.03). Although this seems to be an effect of the 1.0 mg/kg dose, none of the dosages differed significantly from vehicle. Stop-trial performance was significantly impaired (F[3,27]=5.6, p=0.004). Post-hoc comparisons showed that 0.5 mg/kg and 1.0 mg/kg both yielded performance significantly worse compared to vehicle.

Reaction time measures (mRT and SSRT) are depicted in Figure 2. Eltoprazine had no effect on the mRT, or the SSRT. While D-amphetamine significantly decreased mRT at the highest dose (F[3,39]=12.1, p=0.002), the elevation in SSRT did not reach statistical significance. The effects of eltoprazine and D-amphetamine on SSRT (but not mRT) were rate-dependent. In all dosages of eltoprazine a significant but negative correlation was found between SSRT change as a result of drug administration and vehicle SSRT (0.25 mg/kg: r=-0.66, p=0.028; 0.5 mg/kg: r=-0.60, p=0.03; 1.0 mg/kg: r=-0.81, p=0.001). In D-amphetamine, similar results are found at 0.25 mg/kg and 1.0 mg/kg (0.25 mg/kg: r=-0.69, p0=0.028; 1.0 mg/kg, r=-0.61, p=0.016). In other words, animals with a low baseline SSRT displayed a large increase as a result of both eltoprazine and D-amphetamine administration. Rate-dependency of D-amphetamine has been extensively described in literature (e.g. Chiang et al., 2000).

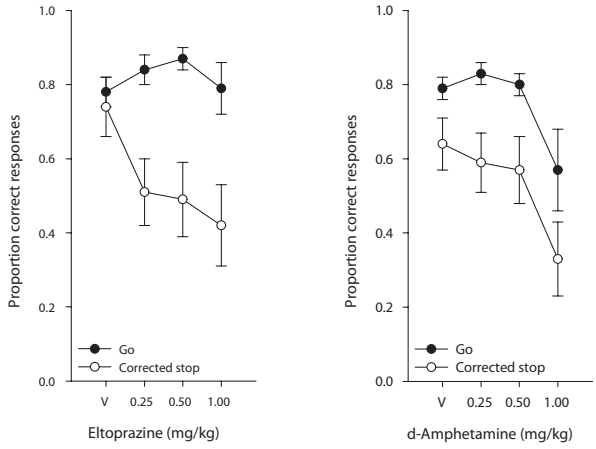

Figure 1 Effect of eltoprazine (left) and D-amphetamine (right) on go and corrected stop success in the stop-signal task. Values are means ± SEM.

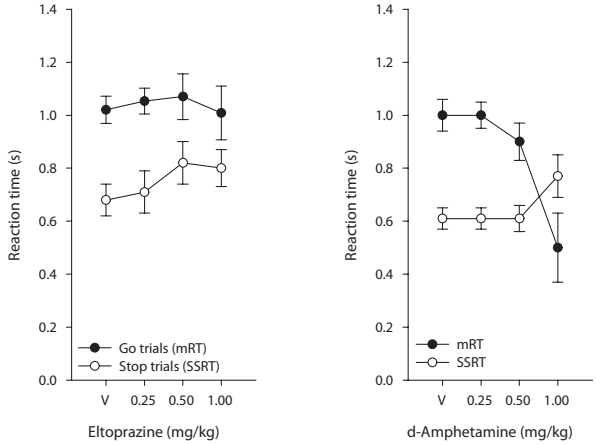

Figure 2 Effect of eltoprazine (left) and D-amphetamine (right) on response time measures of the stop-signal task. Values are means ± SEM.

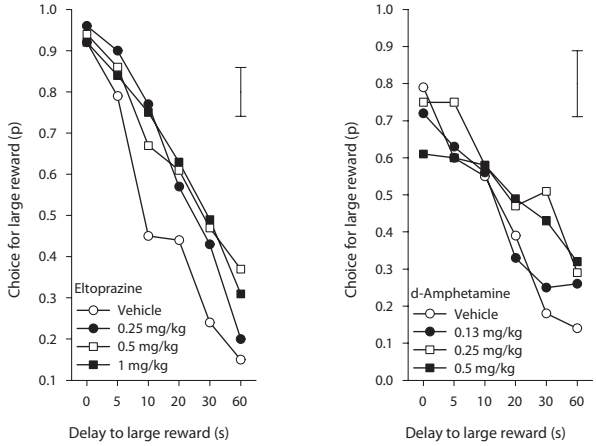

Figure 3 Effect of eltoprazine (left) and D-amphetamine (right) on preference in the delayed reward task. Values represent the mean proportion of choice for the large reward ± SEM.

Eltoprazine	mean	± SEM	D-amphetamine	mean	± SEM
Vehicle	21.1	± 2.9	Vehicle	18.0	± 2.9
0.25 *	30.0	± 3.2	0.25	21.9	± 4.0
0.5 *	32.9	± 3.2	0.5	31.2	± 3.9
1 *	32.8	± 1.8	1	29.3	± 5.1

Table 1 Effect of eltoprazine (left) and D-amphetamine (right) on the area under the preference curve (AUPC) in the delayed reward task. Values represent the means ± SEM. Dosages marked with * indicates a significant increase.

Delayed reward task

Figure 3 displays the choice preference for eltoprazine and D-amphetamine. The area under the preference curve for each of the dosages is listed in Table 1. A high AUPC in the delayed reward task corresponds to low delay aversion. Eltoprazine significantly increased the AUPC, and thus significantly lowered impulsivity in the delayed reward task ($F[3,33]=4.9$, $p=0.007$). All dosages of eltoprazine increased the AUPC. D-amphetamine again displays a similar profile, although the effect was less pronounced, and the increase in AUPC almost reached statistical significance ($F[3,30]=2.7$, $p=0.06$). Post-hoc tests confirmed that this lack of effect was due to the shape of the curve. While 0.25 mg/kg was too low to have any effect, performance was significantly elevated at 0.5 mg/kg, but the effect is lost again at the higher dose of 1.0 mg/kg. The reason for this effect at the highest dose of D-amphetamine seems to be higher variability in the data due to collapsed performance in a subgroup of animals. This may be explained by stereotypy at the highest dose of D-amphetamine.

Preference curves (Figure 3) indicate that all effects of eltoprazine and d-amphetamine are due to a shift to the large reward, while preference at a delay of 0 s remains stable (except at the highest dose of D-amphetamine).

The performance increase by administration of eltoprazine relative to the baseline performance correlated significantly but negatively with baseline performance, indicating rate dependency, which may be due to a ceiling effect (0.25 mg/kg: $r=-0.82$, $p=0.001$; 0.5 mg/kg: $r=-7.64$, $p=0.004$, 1.0 mg/kg: $r=-0.79$, $p=0.002$). A similar but weaker rate-dependency was found for D-amphetamine at the most effective dose (0.5 mg/kg: $r=-0.68$, $p=0.02$).

Microdialysis studies

Effects of eltoprazine

The effects of eltoprazine on dopamine release in the striatum are depicted in Figure 4. As can be seen from that figure, relatively high dosages were needed to measure an increase of dopamine in the striatum compared to the dosages that caused behavioral effects (up to 10 mg/kg for microdialysis compared to a maximum of 1 mg/kg for the behavioral effects). Eltoprazine had a significant elevating effect on dopamine concentrations in the striatum ($F[3,21]=5.2$, $p=0.007$). This effect was exclusively due to the highest dose of eltoprazine (10 mg/kg), as the other dosages did not differ from vehicle. Eltoprazine also has effects on DOPAC release ($F[3,20]=8.1$, $p=0.001$), at the same dose as on dopamine (Figure 5). The slight elevation at 3 mg/kg eltoprazine was not significant.

The effects of eltoprazine on 5-HIAA, a serotonin metabolite, are shown in Figure 6. Eltoprazine causes a significant decrease in 5-HIAA concentrations at all dosages ($F[3,18]=0.001$).

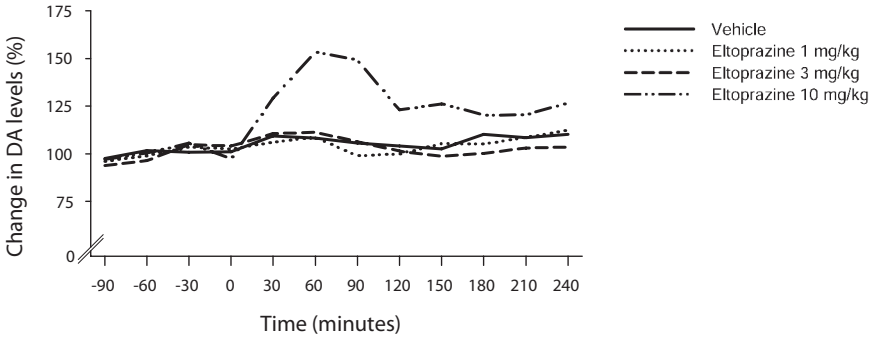

Figure 4 Effects of eltoprazine on dopamine release in the striatum. Values represent mean DA levels in the striatum relative to the average of the 3 baseline samples (-90 to 0 minutes). For reference, the behavioral tests were conducted from 30-60 min (in different animals).

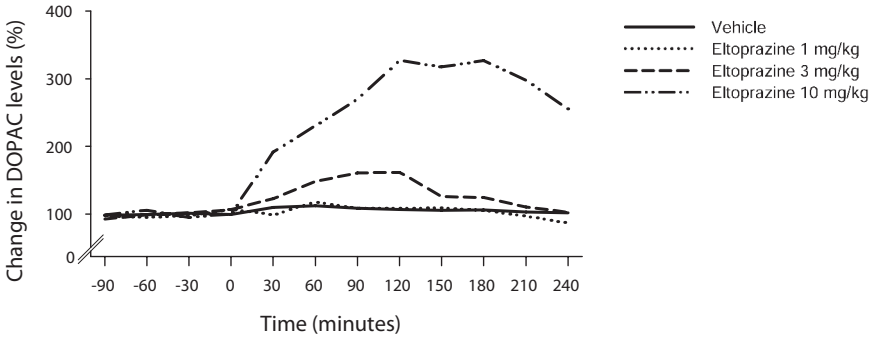

Figure 5 Effects of eltoprazine on DOPAC release in the striatum. Values represent mean DOPAC levels in the striatum relative to the average of the 3 baseline samples (-90 to 0 minutes). For reference, the behavioral tests were conducted from 30-60 min (in different animals).

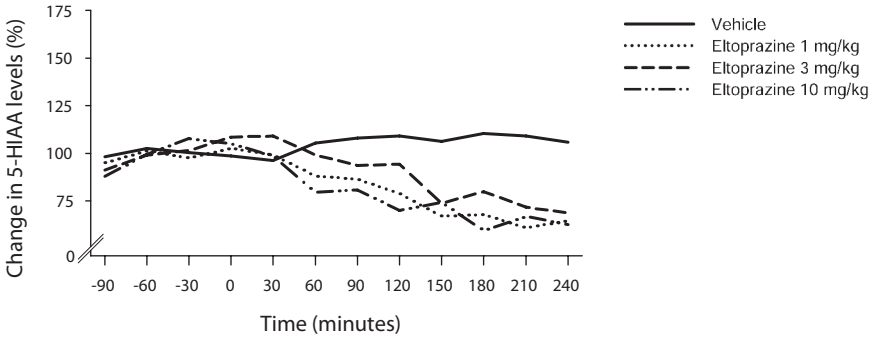

Figure 6 Effects of eltoprazine on 5-HIAA release in the striatum. Values represent mean 5-HIAA levels in the striatum relative to the average of the 3 baseline samples (-90 to 0 minutes). For reference, the behavioral tests were conducted from 30-60 min (in different animals).

Discussion

The current study addressed the possible efficacy of eltoprazine, an anti-aggressive agent and a 5-$HT_{1A/1B}$ receptor agonist (Olivier and van Oorschot, 2005), as an anti-impulsive drug. We tested eltoprazine because of the links between aggressive and impulsive behavior. For example, correlations have been found between delay aversion and aggression in both humans and rats (Ramirez and Andreu, 2005; Solanto et al., 2001; Chapter 3). The results show that eltoprazine, similarly to D-amphetamine, decreased response inhibition in a stop-signal task, but raised inhibition in a delayed reward task. Because the effects of eltoprazine are highly similar to those of D-amphetamine in these impulsivity tasks, it can be postulated that eltoprazine has anti-impulsive properties.

The similarity of the effects of eltoprazine on the two behavioral tasks to D-amphetamine suggests a possible role for dopamine in the effects of eltoprazine in impulsivity. Although eltoprazine did indeed increase dopamine and DOPAC concentrations in the striatum, this effect was only present at a tenfold higher dosage than the behavioral effects. In other words, if the effects of eltoprazine on both types of impulsivity were dopamine-mediated, they probably would have occurred only at those higher dosages. This discrepancy may not be attributed to the relative insensitivity of the in vivo microdialysis technique compared to the behavioral studies, because contrary to eltoprazine, the effects of D-amphetamine in these behavioral tasks are proportional to the rise in dopamine concentrations found using microdialysis studies. Using microdialysis, a significant rise in dopamine concentrations can be demonstrated after administration of D-amphetamine at behaviorally effective dosages (Balcioglu et al., 2003; Dawson et al., 2003; Pothos et al., 1995). At the behaviorally effective dosages of eltoprazine, the 5-HT metabolite 5-HIAA is significantly lowered, reflecting that the presynaptic activity of serotonergic neurons is inhibited and suggesting that the effects of eltoprazine are due to its serotonergic properties.

Eltoprazine is highly selective for 5-HT_{1A} and 5-HT_{1B} receptors, and to a lesser extent, the 5-HT_{2C} receptor (respective K_i values: 50, 52, and 81 nM: Schipper et al., 1990). The 5-HT_{2C} activity proved to be an antagonistic effect and was not relevant for the anti-aggressive effects of eltoprazine (Olivier et al., 1995). The effects of eltoprazine on aggression are ascribed to its effects on 5-HT_{1B} receptors (Olivier and van Oorschot, 2005), although the precise relationship between serotonergic activity and aggression is still a matter of debate (de Boer and Koolhaas, 2005). Through its binding on presynaptic 5-HT_{1A} and 5-HT_{1B}-receptors, eltoprazine causes a decrease in serotonin release. At the same time, however, at the level of postsynaptic neurons containing postsynaptic 5-HT_{1A} and 5-HT_{1B} heteroreceptors, activation of these same receptors mimics (or even hyperstimulates) the normal function of the endogenous ligand serotonin (De Groote et al., 2002). Whether the former or the latter is responsible for the effects of eltoprazine remains speculative, although postsynaptic 5-HT_{1B} heteroreceptors seem very strong candidates (see Olivier and van Oorschot, 2005, for multiple lines of evidence). Since impulsivity in the delayed reward tasks correlates positively with aggressive behavior, and since eltoprazine has in-

hibiting effects in both tasks, we hypothesize that a similar mechanism of eltoprazine underlies both delay aversion and aggression.

The similarity of the behavioral effects of eltoprazine and D-amphetamine is cleary not due to a shared effect on dopamine, and the effects of eltoprazine are hypothesized to be the result of the activation of postsynaptic 5-HT_{1B}-receptors. The question therefore rises whether the effects of D-amphetamine may also be mediated by serotonin. D-amphetamine is not selective for the dopamine transporter, but acts as a monoamine releasing agent and an antagonist at transporters as well, including the norepinephrine and serotonin transporter (Gatley et al., 1996). In the stop-signal task, the effects of D-amphetamine are not indisputably ascribed to dopamine, as human studies have shown that L-dopa does not have an effect on response inhibition (Overtoom et al., 2003). Although noradrenalin is suggested to be involved in response inhibition because of the positive effects of desipramine in the stop-signal task (Overtoom et al., 2003), extensive pharmacology has not ruled out any serotonergic involvement either. In delayed reward tasks, the effects of D-amphetamine are dependent on details of the task used. In the original design used by Evenden and Ryan (1996), the delays were not signaled. In their design, D-amphetamine increased delay aversion. Cardinal and colleagues (2000) found that the effects of D-amphetamine are different if the delay to the large reward is signaled: in signaled procedures, D-amphetamine decreases delay aversion. Van Gaalen and colleagues (2005), however, find stable effects of D-amphetamine in an unsignaled procedure, indicating that signaling may not be as important as previously thought. In the present study, where a similar unsignaled procedure was used, D-amphetamine also significantly reduced delay aversion, but only at specific dosages. The present procedure resembles the procedure used by Van Gaalen and colleagues (2005) most, and in this procedure, the mediation of the effects of D-amphetamine by dopamine has been meticulously proven. First, the selective dopamine transporter antagonist GBR12909 decreases delay aversion (van Gaalen et al., 2005). Second, a behaviorally inert dosage of eticlopride, a selective dopamine D_2-receptor antagonist, attenuated the ability of D-amphetamine to lower delay aversion (van Gaalen et al., 2005). We conclude therefore that, although the behavioral effects of eltoprazine and D-amphetamine are similar, the mechanisms by which they attenuate impulsivity are different.

Having established that both dopamine and serotonin have distinct functions in delay aversion, the question rises whether one of these mechanisms precedes the other in the process that leads to attenuation of delay aversion, or if both mechanisms in parallel lead to changes in a final common pathway. Although the effects of D-amphetamine on delay aversion seem mediated by dopamine (van Gaalen et al., 2005), they seem also to rely on serotonergic neurotransmission: depletion of serotonin by 5,7-DHT attenuates the ability of D-amphetamine to decrease delay aversion (Winstanley et al., 2003). This effect can also be produced by systemic injection of behaviorally inert dosages of the 5-$HT_{1A/7}$-receptor agonist 8-OH-DPAT (Winstanley et al., 2005). Higher dosages of 8-OH-DPAT increased impulsive choice, even after 5,7-DHT-

induced serotonin depletion (Winstanley et al., 2005). These data suggest that a dopaminergic mechanism precedes a serotonergic mechanism in the attenuation of delay aversion, although few studies have been published in which the reverse has been directly addressed.

Because of its unique properties, eltoprazine has several interesting applications in the pharmacotherapy of ADHD. First, comparing the results of eltoprazine and D-amphetamine in the delayed reward task suggest that the therapeutic window for eltoprazine is wider than for D-amphetamine. Second, because of its unique mechanism of action compared to psychostimulants, eltoprazine may be effective in nonresponders to psychostimulant treatment. Estimates of ADHD patients that respond poorly to stimulant treatment range from 70 to 90% (Goldman et al., 1998; Pelham et al., 1999). Third, since eltoprazine combines anti-aggressive effects with anti-impulsive effects, it may be a valuable therapy for ADHD with comorbid oppositional defiant disorder (ODD) or conduct disorder (CD). These disorders are often associated with elevated aggressive behavior (Maughan et al., 2004; Turgay, 2005), and frequently occur together with ADHD: 35% of ODD patients also suffer from ADHD (Bird, 1988), and 30-50% of CD patients have comorbid ADHD (Biederman et al., 1991). Currently, comorbid ADHD/ODD and ADHD/CD are treated with psychostimulants or neuroleptics (Aman et al., 2004; Turgay, 2005). Both treatments have been reported to induce severe side effects (Goldman et al., 1998; Lieberman et al., 2005; Troost et al., 2005), and new therapies are welcome.

Summary, discussion, and perspectives

D

Alice laughed: "There's no use trying,"
she said; "one can't believe impossible things."
"I daresay you haven't had much practice," said the Queen.
"When I was younger, I always did it for half an hour a day.
Why, sometimes I've believed as many as
six impossible things before breakfast."
Lewis Carroll Alice in Wonderland

Summary and Discussion

This thesis describes the search for new drug targets to treat pathological impulsivity. Underlying that search is a theoretical framework consisting of two hypotheses. The first hypothesis states that response inhibition deficits and delay aversion, while independent from each other, both contribute to impulsive behavior. The second hypothesis states that the response selection process underlying response inhibition is also involved in delay aversion, while delay aversion processes do not play a role in response inhibition deficits. In this final chapter, we will discuss the progress that has been made in proving (or falsifying) these hypotheses. Finally, we will discuss the perspectives on future research and the development of new therapies for impulsivity.

Independence of impulsivity subtypes

In the DSM-IV, the manual used for the diagnosis of mental disorders, impulsivity and hyperactivity are listed together in the section on attention-deficit hyperactivity disorder (DSM-IV, 2000). Four ADHD-subtypes are listed: the primarily inattentive type, the hyperactive-impulsive type, the combined type, and ADHD not otherwise specified. To meet the criteria for impulsivity, the following symptoms are listed: (1) Often blurts out answers before questions have been completed. (2) Often has difficulty awaiting turn. (3) Often interrupts or intrudes on others (such as butting into conversations or games). The DSM-IV makes no distinction between impulsivity subtypes, and impulsivity is clustered together with hyperactivity. All symptoms seem to relate to the inhibition of prepotent responses, and no symptoms based on delay aversion are mentioned. Finally, the criteria are judged, not measured, further introducing unreliability to the measurement. We studied the relationship between the impulsivity subtypes and between the impulsivity subtypes and aggressive behavior, sexual behavior, addiction, learning and locomotion using correlations.

The correlational approach

The correlational approach used in Chapter 3 (and to a lesser degree, Chapter 4) has a number of advantages over the standard modeling approach used in Chapter 1. First, this approach gives an idea of what the two impulsivity subtypes actually mean. Second, relationships with extinction and aggression may lead to new insights about extinction and aggression itself. Finally, the approach may aid in the discovery of potential targets for the development of anti-impulsive therapies (Chapter 5 and 6). The correlation coefficient itself may be a good indication of the success one will have in applying therapies for one disorder to another.

The disadvantage of the correlational approach is that nothing is learned about the causality of the constructs. The correlation between aggressive behavior and delay aversion (Chapter 3) does not imply that delay aversion causes aggression. The reverse is also possible, and both delay aversion and aggressive behavior may be caused by a third factor as well. This disadvantage is especially prominent in Chapter 4, where smoking habits are found to be related to delay aversion. To determine whether delay aversion causes a predisposition for a nicotine addiction or

smoking causes delay aversion, a different experiment is necessary in which factors are varied under controlled circumstances. In the modeling approach used in Chapter 1 (using an inbred strain selected for hypertension) causality is also impossible to determine. For example, we cannot conclude that hypertension causes any of the alterations measured in the study described in Chapter 1.

In sum, the used correlation approach is suited as an exploratory strategy, but any relationships should be followed up by controlled experiments.

Correlations between impulsivity subtypes and other behaviors

In Chapter 3, we show that response inhibition and delay aversion are independent constructs. The impulsivity subtypes were quantified in a large group of rats, and none of the measures of response inhibition correlated to delay aversion. In the introduction, we hypothesized how these impulsivity subtypes underlie behavior, including aggression, extinction, and addiction.

We hypothesized that response inhibition was related to extinction of conditioned responses and aggressive behavior. In extinction, a learned response that is no longer rewarded disappears. Extinction does not mean the original response-reward contingency is forgotten; rather, a new memory trace is formed which inhibits the original association (Rescorla, 2004). The description of the competition between the original trace and the extinction trace bears resemblance to the horse-race model of response inhibition (Logan, 1994). This model states that successful inhibition is produced by a disruption of an excitatory process by an independent inhibitory process. In Chapter 3, we see that, contrary to our initial hypothesis, extinction is not related to response inhibition. Moreover, the significant correlation between extinction and delay aversion suggests that the similarities between theories of extinction and response inhibition are coincidental. Instead, we should perhaps interpret persistence in extinction (as found in the SHR in Chapter 1) in terms of delay aversion. Possibly, in animals that display both delay aversion and persistence during extinction the response and the reward are associated more closely. As a result, these animals are unable to properly value responses and delayed rewards, and they also need to create stronger inhibitory traces in extinction tests before they cease their conditioned responses.

Like extinction, we should also revise our view on aggressive behavior. In Chapter 3, we clearly show that aggressive behavior is not related to response inhibition as hypothesized in the introduction. Various measures of aggressive behavior, including the number of bites, fights and wounds on the opponent, were related to delay aversion instead. Different nosologies of aggressive behavior exist (Barratt and Felthous, 2003; Barratt and Slaughter, 1998; Barratt, 1997), and most include 'impulsive aggression'. Impulsive aggression is often seen as an inability to inhibit an aggressive urge. Descriptions such as 'hair-trigger' suggest a deficit in response inhibition (Barratt, 1997), in particular of aggressive urges (a hair-trigger is a firearms trigger modified to respond to very slight pressure). The association between aggressive behavior and

delay aversion suggests that aggressive acts are a result of an altered perception of the balance between immediate rewards and delayed rewards or punishments. Females, food, shelter, or the aggressive act itself serve as the immediate reward (Fish et al., 2005), but what are the long-term rewards or punishments to which these animals are less sensitive? Perhaps not sustaining injuries or advantageous group processes are rewards worth not fighting for.

In Chapter 4, we clearly show that addictive behavior in humans is associated to delay aversion as hypothesized. Addictive behaviors are associated with long-term punishments, but to the addict, these disadvantages are less important than the immediate effects of their addiction.

Development and effects of environment

In Chapter 2, we conducted a longitudinal analysis of both impulsivity subtypes. The results show that response inhibition as measured in the stop task is very stable. In humans, response inhibition is also very stable in adulthood (Bedard et al., 2002; Carver et al., 2001). While changes occur during childhood in humans, the lengthy training time required for the acquisition of the stop-signal task in rats prohibited us from measuring this development. Delay aversion slowly increased over the lifespan. Such changes are not present in humans (Chapter 2; Green et al., 1996), and we hypothesize that this increase may be due to an alteration of the strategy rats use in the delayed reward task rather than actual changed preference. Also in Chapter 2, we show that a radical environmental manipulation, isolation rearing, leads to immediate deficits in response inhibition, while changes in delay aversion are observed only after several months.

The serial process model

The serial-process model as postulated in the introduction to this thesis details the relationship between response inhibition and delay aversion. Not enough experiments were aimed directly at the falsification of the serial process model. Most drugs tested in this thesis were selected for their efficacy in tests for constructs related to the two impulsivity subtypes, rather than to test the predictions made by the serial-process model. Therefore, more research is required. In this section, the various chapters of this thesis are discussed in terms of the serial process model.

Dopamine

The serial process model predicts that since the dopamine system is involved in the valuation of rewards, manipulations of that system have effects on the delayed reward task, but not on the stop-signal task. In the present thesis, the used dopaminergic drugs were D-amphetamine and 7-OH-DPAT, a D_3-receptor agonist. Both drugs do indeed have effects on delay aversion. D-amphetamine significantly lowers delay aversion (Chapter 2 and 5), although this effect does not always reach statistical significance (Chapter 6). In literature, the effects of D-amphetamine are also not entirely consistent. Evenden and Ryan (1996) find that D-amphetamine increases delay aversion, while Cardinal et al. see a reduction under specific test circumstances (2000).

Cardinal et al. report effects of D-amphetamine only if rats are reminded of their choice during the delay with a signal lamp (2000). The results of Van Gaalen et al. (2005), however, show that D-amphetamine can reduce delay aversion even in procedures where the delay is not signaled. In the present thesis, both procedures have been used, although no systematic comparisons have been made. In pilot studies not included in this thesis and in Chapter 2, D-amphetamine significantly lowered delay aversion in unsignaled conditions. In Chapter 2, this effect was only significant in the isolation-reared animals. In Chapter 5, the procedure was signaled, and the effects of D-amphetamine relative to the vehicle condition were larger compared to Chapter 2. In short, many factors may contribute to the efficacy of D-amphetamine in the delayed reward task, including isolation rearing and delay signaling, but none of these factors are required.

The effects of D-amphetamine on delay aversion are supportive of the serial process model, but the model also predicts that dopamine is not very important in response inhibition. The results of Chapter 2 clearly show that administration of D-amphetamine dose-dependently worsens response inhibition. A similar effect is found in Chapter 6, where 1 mg/kg D-amphetamine increases the speed of the go-response. Either the effects of D-amphetamine are caused by antagonism of the noradrenalin or serotonin transporters, or the serial process model needs revision. Perhaps the effects of dopamine on the motor system (currently outside the domain of the serial process model) need to be considered.

The D_3-receptor agonist 7-OH-DPAT decreased choice for the large reward, but only at a specific dose. In future research, D_3-receptor antagonists should be examined, for they might have a potential therapeutic value in impulsivity.

Serotonin

The serial-process model predicts that, since the serotonin system is involved in response selection, manipulations of the serotonin system will result in altered response inhibition, but not in changes in delay aversion. In this thesis, several serotonergic drugs are tested. Fluvoxamine, a serotonin transporter antagonist, has modest effects in both the stop-signal task and the delayed reward task. The interaction between fluvoxamine and rearing conditions make interpretation difficult. In addition, the brain has a rich variety of serotonin receptors, and fluvoxamine, through its action on the serotonin transporter, activates them all.

Prompted by the links between aggressive behavior and delay aversion (Chapter 3), we tested eltoprazine in both the stop-signal task (Chapter 6) and the delayed reward task (Chapter 5 and 6). Eltoprazine is an anti-aggressive drug with a high affinity for 5-HT_{1B} and 5-HT_{1A}-receptors. As hypothesized in the introduction to this thesis, a relationship should exist between aggressive behavior and response inhibition. Therefore, eltoprazine was predicted to be effective in the stop-signal task. The results of Chapter 3, however, show that aggressive behavior is linked to delay aversion instead of response inhibition, and eltoprazine can thus be hypothesized to be effective in the delayed reward task instead. In a direct comparison between the effects of elto-

prazine in the delayed reward task and the stop-signal task, we find that eltoprazine decreases response inhibition in the stop-signal task, but increases choice for the large reward. The decrease in response inhibition may be the result of binding to the 5-HT_{1B}-autoreceptor, thereby lowering available serotonin, and thus impairing response selection. The effects of eltoprazine on delay aversion, however, are unexpected considering the serial process model. One possible reason eltoprazine increases choice for the large reward, is that a lack of available serotonin through activation of presynaptic 5-HT receptors results in cognitive inflexibility, making the rats stick to the responses made in the first block. Since the delay is 0 s in the first block, inflexibility leads to an advantage for the lever that delivers the large reward. The effects of eltoprazine are much stronger in Chapter 6 than they are in Chapter 5. This is reminiscent of the unstable effects of D-amphetamine described above, and more research is required to determine the optimal conditions for measuring drug effects in the delayed reward task.

5-HT_{1A}-receptor agonists, such as flesinoxan (Chapter 5) and 8-OH-DPAT (Evenden, 1999a), lower choice for the large reward even at 0 s delays. Further, 8-OH-DPAT has been found to increase choice for the large reward at longer delays (Evenden, 1999a). This indifference to the delay may also be a result of cognitive inflexibility. The decreased choice for the large reward at a delay of 0 s, however, indicates the processes underlying the effects of 5-HT_{1A}-receptor agonists and eltoprazine are not identical.

Finally, a number of serotonin receptor antagonists, including GR127935, a 5-HT_{1B}-receptor antagonist (Chapter 5), had no effects on delay aversion (Evenden, 1999a), indicating that delay aversion (or any of the other processes involved in execution of the delayed reward task) are not under tonic serotonergic control.

Perspectives

From the above discussion, it is clear that more research is required. This final section serves to summarize the directions future research should take.

Optimization of instruments

As illustrated above, it is unclear what factors contribute to the contradictory results sometimes found in the delayed reward task. In addition, the stop-signal task is a very cumbersome task that takes several months of training. Both tasks need optimization, and direct comparisons of task variations should be conducted before new, large-scale experiments are planned.

As described in this thesis, serotonergic manipulations may interact with execution of tasks through a response selection mechanism. Therefore, the delays used in the delayed reward tasks should be presented both in ascending and random (or descending) orders. Then, it will become clear if a manipulation has effects on the ability to switch between alternatives. In its current form, response-switching problems lead to an over-estimation of the anti-impulsive potential of a drug, since the animal will keep responding as if the choice were between a large delay and a small delay, both available immediately.

Reducing training time for the stop-task by at least a month would make the task much more useful. It should be noted, however, that the stable performance of rats in the stop-task in its current form is a big advantage. Perhaps premature responses in the five-choice serial reaction time task (which requires shorter training times) are also a good measurement of response inhibition, but this hypothesis needs much more work. A correlational approach similar to that used in Chapter 3 could be used to study the similarities between the two tasks.

Classification of disorders

By determining the contribution of response inhibition deficits and delay aversion to various mental disorders, new therapies could be developed. In particular, ADHD is a very broad diagnosis including patients suffering from various types of impulsivity. The current division of ADHD patients into subtypes has little theoretical basis (Evenden, 1999b; Nigg et al., 2005). Perhaps a new classification system based on response inhibition deficits and delay aversion proves useful in the prediction of non-response and in the development of new, more selective therapies.

Basic research into the serial-process model

Although response inhibition deficits and delay aversion are useful constructs, not nearly enough is known about their similarities and differences. As described above, several problems have risen with the strict separation of neurotransmitter systems as hypothesized in the serial-process model described in the introduction. For example, motor effects of dopamine are not considered, and neither is the involvement of serotonin in motivational processes. We conclude that the fundaments of the model still stand, but the model requires more research. In particu-

lar, we should study how widely applicable the role of dopamine and serotonin as hypothesized in the model actually is.

A research program (given optimized tasks as advised above) should focus on pharmacology and neuroanatomy of both impulsivity subtypes. More insight is needed into the serotonin and dopamine systems in the delayed reward task and the stop-task. Only a small subset of all receptors has been examined. Further, lesions studies and experiments with local infusions of selective agonists and antagonists could uncover which brain areas are involved in both impulsivity subtypes.

Mechanism of active drugs

Finally, two potential targets were identified in the current thesis, and more research into the involvement of those targets is required. The first and most extensively studied target in this thesis is the 5-HT_{1B}-receptor (Chapter 6). To study the effects of eltoprazine, we can use the fact that central serotonin depletion has very few effects on delay aversion. If eltoprazine has effects in serotonin-depleted animals, the effects of eltoprazine may be attributed to post-synaptic 5-HT_{1B}-receptors. The second target is the dopamine D_3-receptor. In particular, inhibition of this receptor may lower delay aversion. Co-administration of eltoprazine and a dopamine D_3-receptor antagonist may provide a maximum effect with minimal side-effects.

Appendix A
The stop-signal task

The stop-signal reaction time task

In the rat version of the stop signal reaction time task, rats are trained to press a lever (Figure 1). Immediately after this response, a second lever is presented. In go-trials, responding on this lever (Figure 2a) delivers a food reward (Figure 3a). In stop-trials, a tone presented either simultaneously with the extraction of the second lever, or a short while later (Figure 2b). In response to this tone, the rat should withhold its response on the second lever, which results in the delivery of a food reward (Figure 3b). Failure to respond on the second lever during go-trials, or failure to inhibit responding during stop-trials both results in a time-out: the houselight is turned off, and for 5 s, no new trials are presented.

Measures in the stop-signal reaction time task

We obtain four important measures from the stop-signal reaction time task. The first is the percentage of correct responses in go-trials. The second is the percentage of correct responses in stop-trials, but this measure is corrected for the number of omissions made in the go-trials (Equation 1). This correction provides a better estimate of the trials in which rats were actually inhibiting their response, rather then just not responding.

The third measure is the mean reaction time on go-trials, the mRT. Using the frequency distribution of mRTs, a fourth measure can be calculated: the stop-signal reaction time (SSRT). In the horse-race model, response inhibition is described as a race between an excitatory and an inhibitory signal (Logan, 1994). If the excitatory signal wins, the response is executed. If the inhibitory signal wins, however, it disrupts the excitatory signal and the response is inhibited. The speed of the inhibitory process (the SSRT) must be estimated, since successful inhibition does not result in a response, and thus no reaction time can be measured. To estimate the SSRT, the mRT distribution is plotted, and the percentile of the distribution that equals the proportion of failed stops is taken. This speed has to be corrected for the onset of the stop-stimulus relative to the go-stimulus (Figure 4). This method assumes independence of the excitatory and inhibitory signals.

$$P_i' = \frac{P_i - (P_o \times P_i)}{1 - (P_o \times P_i)}$$

Equation 1 Method used to correct the raw inhibition probability for omissions made during go-trials. P_i' is the corrected inhibition probability, P_i is the raw inhibition probability in stop trials, and P_o is probability of omissions in go-trials.

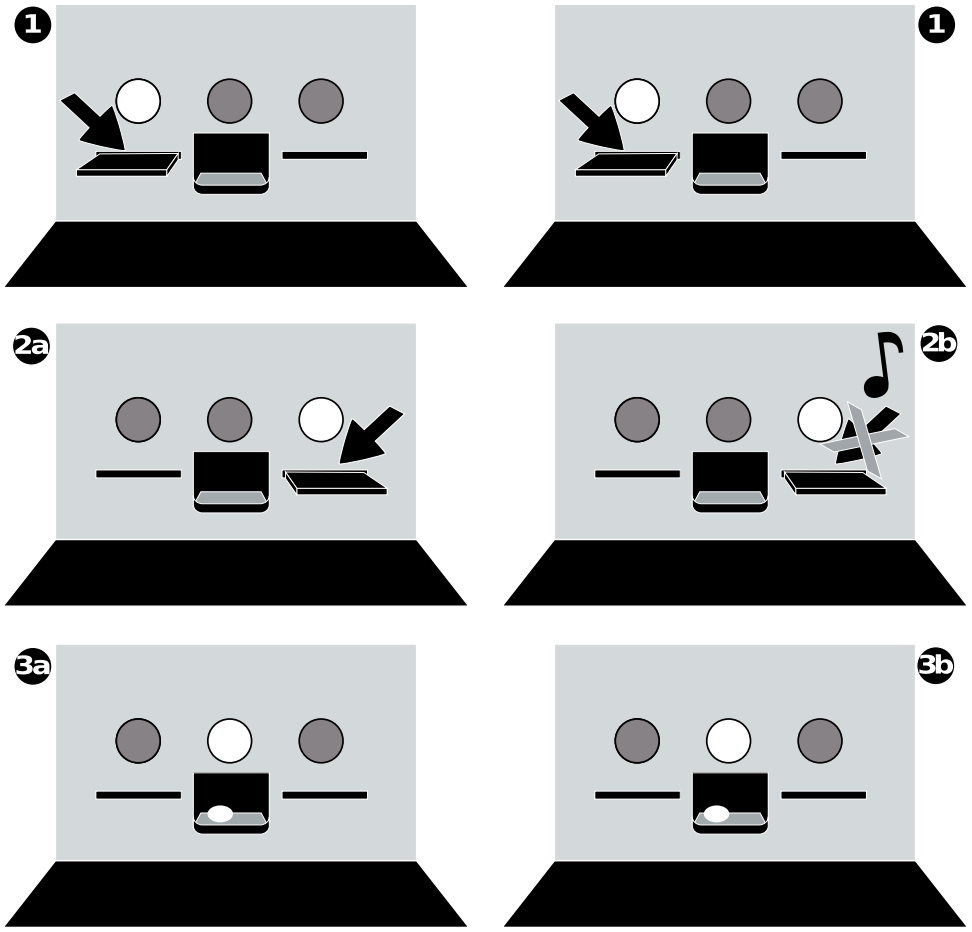

Figure 1-3 Description of the stop-signal task

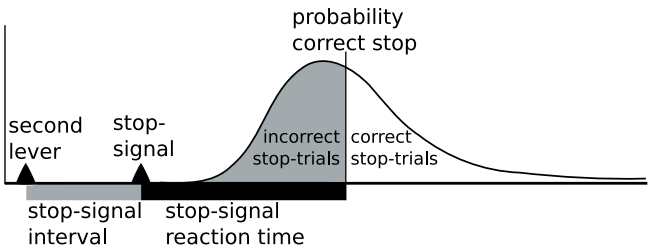

Figure 4 Schematic overview of some of the parameters of the rat stop-task.

Appendix B
The delayed reward task

The delayed reward task

In the delayed reward task, rats repeatedly make a choice between a small, immediately available reward, and a large reward available after a delay. A delayed reward task session is divided into five (Chapter 5) or six (Chapter 2, 3 and 6) blocks. In the first block, the delay is short (0 s), but in the final blocks, the delay is long (40 to 60 s). The blocks are divided into trials. The first two trials of each block are forced choice trials, in which only one lever is present. The remaining trials are choice trials, in which both levers are presented. In choice trials, both levers are extended into the box (Figure 1). A response on one lever (Figure 2a) delivers a single food reward (Figure 3a), while a response on the other lever (Figure 2b) results in a delay (Figure D), and four food rewards (Figure 3b).

Measures of the delayed reward task

Waiting for a reward diminishes its value. If there is no delay, the subjective value of a reward is maximal. At the indifference point, the delay decreased the value of the large reward to the same value as the small reward (Figure 4). At longer delays, the smaller reward has a higher subjective value than the larger reward.

Several different models have been proposed to describe the speed by which delays affect subjective value, and a growing body of evidence now favors a hyperbolic over an exponential account of the data (Vuchinich and Simpson, 1998). The hyperbolic function (Equation 1, Mazur, 1997) predicts the subjective value of a reinforcer (V*), considering the initial value (V), and the delay (D). Individual differences in discounting magnitude are expressed in the discounting constant k. The discounting constant is often calculated for individuals, and may signify an important personality trait, with value of k being higher in impulsive individuals.

Calculation of hyperbolic curves for individuals is not without problems, and there are no criteria labeling a fit as "good". Articles claiming good fits report R2 values as high as 0.99 (Petry, 2001) and some report values lower than 0.80 (Crean et al., 2002; Ortner et al., 2003; Reynolds et al., 2004). Because of these problems, some researchers suggest a regression solution with a second parameter: an exponent s for the divisor (Equation 2). The two-factor model is especially useful to fit individual data, as individual choices are often not described very well by Equation 1 (Green et al., 2004; Holt et al., 2003). The two-factor model necessarily creates a better fit for sample data as well, but added value is small (for example, see Vuchinich and Simpson, 1998: 87% explained variance for the one-factor model, 94% explained variance for the two-factor model). Because of the difficulties associated with curve-fitting, and the effects curve-fitting has on inter-subject variation, some researchers advise to calculate the area under the preference curve as a theory-neutral measure of impulsivity (Holt et al., 2003; Myerson et al., 2001). This approach is therefore used in the current thesis.

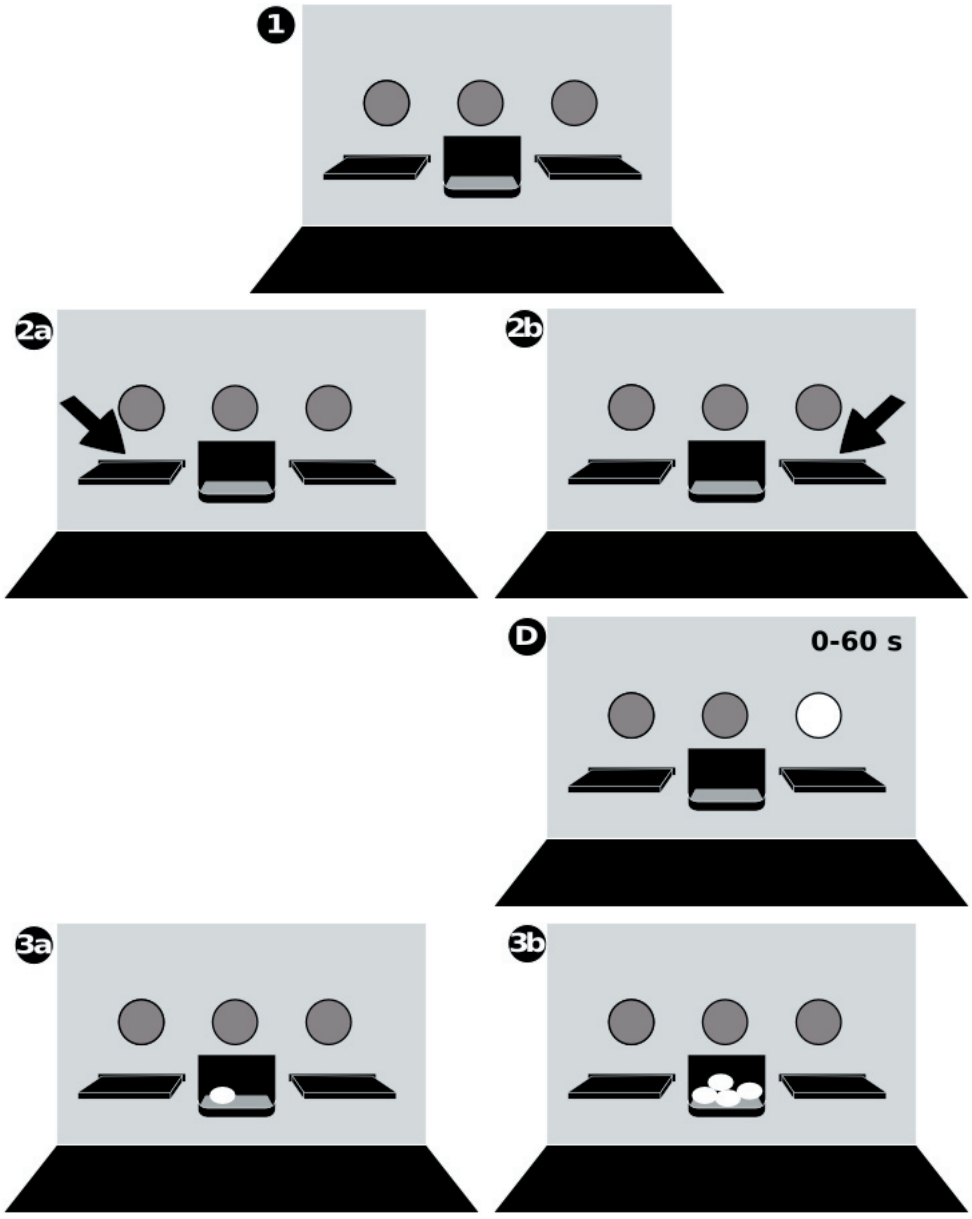

Figure 1-3 Description of the delayed reward task.

$$V^* = \frac{V}{1 + k \times D}$$

Equation 1 Subjective reward value as a function of the delay to the availability of that reward. V* is the subjective value, V is the actual reward value, D is the delay to the reward, and k is an individual constant.

$$V^* = \frac{V}{(1 + k \times D)^s}$$

Equation 2 Subjective reward value as a function of the delay to the availability of that reward. This equation contains an additional parameter s, which is an individual constant.

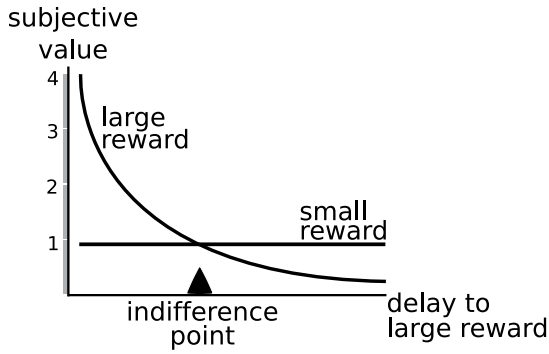

Figure 4 Figure illustrating the diminishing of the delayed reward with increasing delays. Without delay, the value of the rewards is maximal. The longer the delay, the less the reward is worth, until it is worth less than the smaller reward. The intersection is the indifference point.

References

Acosta JI, Boynton FA, Kirschner KF and Neisewander JL. Stimulation of 5-HT1B receptors decreases cocaine- and sucrose-seeking behavior. Pharmacol Biochem Behav 2005; 80: 297-307.

Adriani W and Laviola G. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. Behav Pharmacol 2004; 15: 341-52.

Adriani W, Spijker S, Deroche-Gamonet V, Laviola G, Le Moal M, Smit AB and Piazza PV. Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats. J Neurosci 2003; 23: 4712-6.

Ahmed SH, Stinus L, Le Moal M and Cador M. Social deprivation enhances the vulnerability of male Wistar rats to stressor- and amphetamine-induced behavioral sensitization. Psychopharmacology (Berl) 1995; 117: 116-24.

Ainslie G. Specious reward: a behavioral theory of impulsiveness and impulse control. Psychol Bull 1975; 82: 463-96.

Albrecht B, Banaschewski T, Brandeis D, Heinrich H and Rothenberger A. Response inhibition deficits in externalizing child psychiatric disorders: An ERP-study with the Stop-task. Behav Brain Funct 2005; 1: 22.

Alessi SM and Petry NM. Pathological gambling severity is associated with impulsivity in a delay discounting procedure. Behav Processes 2003; 64: 345-354.

Aman MG, Binder C and Turgay A. Risperidone effects in the presence/absence of psychostimulant medicine in children with ADHD, other disruptive behavior disorders, and subaverage IQ. J Child Adolesc Psychopharmacol 2004; 14: 243-54.

Amieva H, Phillips LH, Della Sala S and Henry JD. Inhibitory functioning in Alzheimer's disease. Brain 2004; 127: 949-64.

Andreoli M, Tessari M, Pilla M, Valerio E, Hagan JJ and Heidbreder CA. Selective antagonism at dopamine D3 receptors prevents nicotine-triggered relapse to nicotine-seeking behavior. Neuropsychopharmacology 2003; 28: 1272-80.

Aron AR, Dowson JH, Sahakian BJ and Robbins TW. Methylphenidate improves response inhibition in adults with attention-deficit/hyperactivity disorder. Biol Psychiatry 2003; 54: 1465-8.

Aron AR and Poldrack RA. The cognitive neuroscience of response inhibition: relevance for genetic research in attention-deficit/hyperactivity disorder. Biol Psychiatry 2005; 57: 1285-92.

Ashby CR, Jr., Paul M, Gardner EL, Heidbreder CA and Hagan JJ. Acute administration of the selective D3 receptor antagonist SB-277011A blocks the acquisition and expression of the conditioned place preference response to heroin in male rats. Synapse 2003; 48: 154-6.

Badcock JC, Michie PT, Johnson L and Combrinck J. Acts of control in schizophrenia: dissociating the components of inhibition. Psychol Med 2002; 32: 287-97.

Bakshi VP and Geyer MA. Ontogeny of isolation rearing-induced deficits in sensorimotor gating in rats. Physiol Behav 1999; 67: 385-92.

Balcells-Olivero M, Cousins MS and Seiden LS. Holtzman and Harlan Sprague-Dawley rats: differences in DRL 72-sec performance and 8-hydroxy-di-propylamino tetralin-induced hypothermia. J Pharmacol Exp Ther 1998; 286: 742-52.

Balcells-Olivero M, Richards JB and Seiden LS. Sensitization to amphetamine on the differential-reinforcement-of-low-rate 72-s schedule. Psychopharmacology (Berl) 1997; 133: 207-13.

Balcioglu A, Zhang K and Tarazi FI. Dopamine depletion abolishes apomorphine- and amphetamine-induced increases in extracellular serotonin levels in the striatum of conscious rats: a microdialysis study. Neuroscience 2003; 119: 1045-53.

Band GP and van Boxtel GJ. Inhibitory motor control in stop paradigms: review and reinterpretation of neural mechanisms. Acta Psychol (Amst) 1999; 101: 179-211.

Barkley RA. Response Inhibition in Attention-Deficit Hyperactivity Disorder. Mental Retardation and Developmental Disabilities 1999; 5: 177-184.

Barkley RA, Edwards G, Laneri M, Fletcher K and Metevia L. Executive functioning, temporal discounting, and sense of time in adolescents with attention deficit hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD). J Abnorm Child Psychol 2001; 29: 541-56.

Barnes NM and Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology 1999; 38: 1083-152.

Barratt ES and Felthous AR. Impulsive versus premeditated aggression: implications for mens rea decisions. Behav Sci Law 2003; 21: 619-30.

Barratt ES and Slaughter L. Defining, measuring, and predicting impulsive aggression: a heuristic model. Behav Sci Law 1998; 16: 285-302.

Barratt ES, Stanford MS, Felthous AR and Kent TA. The effects of phenytoin on impulsive and premeditated aggression: a controlled study. J Clin Psychopharmacol 1997; 17: 341-9.

Baunez C and Robbins TW. Effects of dopamine depletion of the dorsal striatum and further interaction with subthalamic nucleus lesions in an attentional task in the rat. Neuroscience 1999; 92: 1343-56.

Bedard AC, Nichols S, Barbosa JA, Schachar R, Logan GD and Tannock R. The development of selective inhibitory control across the life span. Dev Neuropsychol 2002; 21: 93-111.

Bekker EM, Overtoom CC, Kenemans JL, Kooij JJ, De Noord I, Buitelaar JK and Verbaten MN. Stopping and changing in adults with ADHD. Psychol Med 2005; 35: 807-16.

Belzung C, Le Guisquet AM, Barreau S and Calatayud F. An investigation of the mechanisms responsible for acute fluoxetine-induced anxiogenic-like effects in mice. Behav Pharmacol 2001; 12: 151-62.

Beninger RJ and Phillips AG. Possible involvement of serotonin in extinction. Pharmacol Biochem Behav 1979; 10: 37-41.

Bickel WK, Odum AL and Madden GJ. Impulsivity and cigarette smoking: delay discounting in current, never, and ex-smokers. Psychopharmacology (Berl) 1999; 146: 447-54.

Biederman J, Newcorn J and Sprich S. Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. Am J Psychiatry 1991; 148: 564-77.

Bizarro L, Patel S, Murtagh C and Stolerman IP. Differential effects of psychomotor stimulants on attentional performance in rats: nicotine, amphetamine, caffeine and methylphenidate. Behav Pharmacol 2004; 15: 195-206.

Bizot J, Le Bihan C, Puech AJ, Hamon M and Thiebot M. Serotonin and tolerance to delay of reward in rats. Psychopharmacology (Berl) 1999; 146: 400-12.

Bizot JC, Thiebot MH, Le Bihan C, Soubrie P and Simon P. Effects of imipramine-like drugs and serotonin uptake blockers on delay of reward in rats. Possible implication in the behavioral mechanism of action of antidepressants. J Pharmacol Exp Ther 1988; 246: 1144-51.

Bolanos CA, Barrot M, Berton O, Wallace-Black D and Nestler EJ. Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. Biol Psychiatry 2003; 54: 1317-29.

Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B and Schwartz JC. Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. Brain Res 1991; 564: 203-19.

Bouwknecht JA, Hijzen TH, van der Gugten J, Maes RA, Hen R and Olivier B. Absence of 5-HT(1B) receptors is associated with impaired impulse control in male 5-HT(1B) knockout mice. Biol Psychiatry 2001; 49: 557-68.

Broersen LM and Uylings HB. Visual attention task performance in Wistar and Lister hooded rats: response inhibition deficits after medial prefrontal cortex lesions. Neuroscience 1999; 94: 47-57.

Brunner D and Hen R. Insights into the neurobiology of impulsive behavior from serotonin receptor knockout mice. Ann N Y Acad Sci 1997; 836: 81-105.

Bull E, Reavill C, Hagan JJ, Overend P and Jones DN. Evaluation of the spontaneously hypertensive rat as a model of attention deficit hyperactivity disorder: acquisition and performance of the DRL-60s test. Behav Brain Res 2000; 109: 27-35.

Cador M, Robbins TW and Everitt BJ. Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. Neuroscience 1989; 30: 77-86.

Cardinal RN, Daw N, Robbins TW and Everitt BJ. Local analysis of behaviour in the adjusting-delay task for assessing choice of delayed reinforcement. Neural Netw 2002; 15: 617-34.

Cardinal RN and Everitt BJ. Neural and psychological mechanisms underlying appetitive learning: links to drug addiction. Curr Opin Neurobiol 2004; 14: 156-62.

Cardinal RN, Pennicott DR, Sugathapala CL, Robbins TW and Everitt BJ. Impulsive choice induced in rats by lesions of the nucleus accumbens core. Science 2001; 292: 2499-501.

Cardinal RN, Robbins TW and Everitt BJ. The effects of d-amphetamine, chlordiazepoxide, al-

pha-flupenthixol and behavioural manipulations on choice of signalled and unsignalled delayed reinforcement in rats. Psychopharmacology (Berl) 2000; 152: 362-75.

Cardinal RN, Winstanley CA, Robbins TW and Everitt BJ. Limbic corticostriatal systems and delayed reinforcement. Ann N Y Acad Sci 2004; 1021: 33-50.

Carli M, Robbins TW, Evenden JL and Everitt BJ. Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. Behav Brain Res 1983; 9: 361-80.

Carver AC, Livesey DJ and Charles M. Age related changes in inhibitory control as measured by stop signal task performance. Int J Neurosci 2001; 107: 43-61.

Casey BJ, Castellanos FX, Giedd JN, Marsh WL, Hamburger SD, Schubert AB, Vauss YC, Vaituzis AC, Dickstein DP, Sarfatti SE and Rapoport JL. Implication of right frontostriatal circuitry in response inhibition and attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 1997; 36: 374-83.

Chiang TJ, Al-Ruwaitea AS, Mobini S, Ho MY, Bradshaw CM and Szabadi E. The effect of d-amphetamine on performance on two operant timing schedules. Psychopharmacology (Berl) 2000; 150: 170-84.

Clarke HF, Dalley JW, Crofts HS, Robbins TW and Roberts AC. Cognitive inflexibility after prefrontal serotonin depletion. Science 2004; 304: 878-80.

Coffey SF, Gudleski GD, Saladin ME and Brady KT. Impulsivity and rapid discounting of delayed hypothetical rewards in cocaine-dependent individuals. Exp Clin Psychopharmacol 2003; 11: 18-25.

Cole BJ and Robbins TW. Amphetamine impairs the discriminative performance of rats with dorsal noradrenergic bundle lesions on a 5-choice serial reaction time task: new evidence for central dopaminergic-noradrenergic interactions. Psychopharmacology (Berl) 1987; 91: 458-66.

Corman SL, Fedutes BA and Culley CM. Atomoxetine: the first nonstimulant for the management of attention-deficit/hyperactivity disorder. Am J Health Syst Pharm 2004; 61: 2391-9.

Crean J, Richards JB and de Wit H. Effect of tryptophan depletion on impulsive behavior in men with or without a family history of alcoholism. Behav Brain Res 2002; 136: 349-57.

Dalen L, Sonuga-Barke EJ, Hall M and Remington B. Inhibitory deficits, delay aversion and preschool AD/HD: implications for the dual pathway model. Neural Plast 2004; 11: 1-11.

Dalley JW, Theobald DE, Pereira EA, Li PM and Robbins TW. Specific abnormalities in serotonin release in the prefrontal cortex of isolation-reared rats measured during behavioural performance of a task assessing visuospatial attention and impulsivity. Psychopharmacology (Berl) 2002; 164: 329-40.

Damsma G, Bottema T, Westerink BH, Tepper PG, Dijkstra D, Pugsley TA, MacKenzie RG, Heffner TG and Wikstrom H. Pharmacological aspects of R-(+)-7-OH-DPAT, a putative dopamine D3 receptor ligand. Eur J Pharmacol 1993; 249: R9-10.

Damsma G, Pfaus JG, Wenkstern D, Phillips AG and Fibiger HC. Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male rats: comparison with novelty and locomotion. Behav Neurosci 1992; 106: 181-91.

Davids E, Zhang K, Tarazi FI and Baldessarini RJ. Animal models of attention-deficit hyperactivity disorder. Brain Res Brain Res Rev 2003; 42: 1-21.

Dawson LA, Nguyen HQ and Li P. Potentiation of amphetamine-induced changes in dopamine and 5-HT by a 5-HT(6) receptor antagonist. Brain Res Bull 2003; 59: 513-21.

de Boer SF and Koolhaas JM. 5-HT(1A) and 5-HT(1B) receptor agonists and aggression: A pharmacological challenge of the serotonin deficiency hypothesis. Eur J Pharmacol 2005; 526: 125-39.

de Boer SF, Lesourd M, Mocaer E and Koolhaas JM. Selective antiaggressive effects of alnespirone in resident-intruder test are mediated via 5-hydroxytryptamine1A receptors: A comparative pharmacological study with 8-hydroxy-2-dipropylaminotetralin, ipsapirone, buspirone, eltoprazine, and WAY-100635. J Pharmacol Exp Ther 1999; 288: 1125-33.

De Bruin NM, Kiliaan AJ, De Wilde MC and Broersen LM. Combined uridine and choline administration improves cognitive deficits in spontaneously hypertensive rats. Neurobiol Learn Mem 2003; 80: 63-79.

De Groote L, Olivier B and Westenberg HG. Extracellular serotonin in the prefrontal cortex is limited through terminal 5-HT(1B) autoreceptors: a microdialysis study in knockout mice. Psychopharmacology (Berl) 2002; 162: 419-24.

De Jong R, Coles MG and Logan GD. Strategies and mechanisms in nonselective and selective inhibitory motor control. J Exp Psychol Hum Percept Perform 1995; 21: 498-511.

de Wit H, Enggasser JL and Richards JB. Acute administration of d-amphetamine decreases impulsivity in healthy volunteers. Neuropsychopharmacology 2002; 27: 813-25.

Di Ciano P and Everitt BJ. Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. J Neurosci 2004; 24: 7167-73.

Diana G. Does hypertension alone lead to cognitive decline in spontaneously hypertensive rats? Behav Brain Res 2002; 134: 113-21.

Dimitrov M, Nakic M, Elpern-Waxman J, Granetz J, O'Grady J, Phipps M, Milne E, Logan GD, Hasher L and Grafman J. Inhibitory attentional control in patients with frontal lobe damage. Brain Cogn 2003; 52: 258-70.

Domeney A and Feldon J. The disruption of prepulse inhibition by social isolation in the Wistar rat: how robust is the effect? Pharmacol Biochem Behav 1998; 59: 883-90.

DSM-IV. Diagnostic and Statistical Manual of Mental Disorders, 4th ed., Text Revision. American Psychiatric Association, 2000.

Eagle DM and Robbins TW. Inhibitory control in rats performing a stop-signal reaction-time task: effects of lesions of the medial striatum and d-amphetamine. Behav Neurosci 2003a; 117: 1302-17.

Eagle DM and Robbins TW. Lesions of the medial prefrontal cortex or nucleus accumbens core do not impair inhibitory control in rats performing a stop-signal reaction time task. Behav Brain Res 2003b; 146: 131-44.

Einon DF and Morgan MJ. Early isolation produces enduring hyperactivity in the rat, but no effect upon spontaneous alternation. Q J Exp Psychol 1978; 30: 151-6.

Elia J, Borcherding BG, Rapoport JL and Keysor CS. Methylphenidate and dextroamphetamine treatments of hyperactivity: are there true nonresponders? Psychiatry Res 1991; 36: 141-55.

Evenden J and Meyerson B. The behavior of spontaneously hypertensive and Wistar Kyoto rats under a paced fixed consecutive number schedule of reinforcement. Pharmacol Biochem Behav 1999; 63: 71-82.

Evenden JL. The pharmacology of impulsive behaviour in rats VII: the effects of serotonergic agonists and antagonists on responding under a discrimination task using unreliable visual stimuli. Psychopharmacology (Berl) 1999a; 146: 422-31.

Evenden JL. Varieties of impulsivity. Psychopharmacology (Berl) 1999b; 146: 348-61.

Evenden JL and Ryan CN. The pharmacology of impulsive behaviour in rats: the effects of drugs on response choice with varying delays of reinforcement. Psychopharmacology (Berl) 1996; 128: 161-70.

Evenden JL and Ryan CN. The pharmacology of impulsive behaviour in rats VI: the effects of ethanol and selective serotonergic drugs on response choice with varying delays of reinforcement. Psychopharmacology (Berl) 1999; 146: 413-21.

Falls WA, Miserendino MJ and Davis M. Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. J Neurosci 1992; 12: 854-63.

Feola TW, de Wit H and Richards JB. Effects of d-amphetamine and alcohol on a measure of behavioral inhibition in rats. Behav Neurosci 2000; 114: 838-48.

Ferguson SA and Cada AM. A longitudinal study of short- and long-term activity levels in male and female spontaneously hypertensive, Wistar-Kyoto, and Sprague-Dawley rats. Behav Neurosci 2003; 117: 271-82.

Ferguson SA and Cada AM. Spatial learning/memory and social and nonsocial behaviors in the Spontaneously Hypertensive, Wistar-Kyoto and Sprague-Dawley rat strains. Pharmacol Biochem Behav 2004; 77: 583-94.

Ferguson SA and Gray EP. Aging effects on elevated plus maze behavior in spontaneously hypertensive, Wistar-Kyoto and Sprague-Dawley male and female rats. Physiol Behav 2005; 85: 621-8.

Ferguson SA, Gray EP and Cada AM. Early behavioral development in the spontaneously hypertensive rat: a comparison with the Wistar-Kyoto and Sprague-Dawley strains. Behav Neurosci 2003; 117: 263-70.

Festing MF and Bender K. Genetic relationships between inbred strains of rats. An analysis based on genetic markers at 28 biochemical loci. Genet Res 1984; 44: 271-81.

Fillmore MT, Kelly TH and Martin CA. Effects of d-amphetamine in human models of information processing and inhibitory control. Drug Alcohol Depend 2005; 77: 151-9.

Fish EW, DeBold JF and Miczek KA. Escalated aggression as a reward: corticosterone and GABA(A) receptor positive modulators in mice. Psychopharmacology (Berl) 2005; 182: 116-27.

Fletcher PJ, Azampanah A and Korth KM. Activation of 5-HT(1B) receptors in the nucleus accumbens reduces self-administration of amphetamine on a progressive ratio schedule. Pharmacol Biochem Behav 2002; 71: 717-25.

Fletcher PJ and Korth KM. Activation of 5-HT1B receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward. Psychopharmacology (Berl) 1999; 142: 165-74.

Gainetdinov RR and Caron MG. An animal model of attention deficit hyperactivity disorder. Mol Med Today 2000; 6: 43-4.

Gao G, Wang X, He S, Li W, Wang Q, Liang Q, Zhao Y, Hou F, Chen L and Li A. Clinical study for alleviating opiate drug psychological dependence by a method of ablating the nucleus accumbens with stereotactic surgery. Stereotact Funct Neurosurg 2003; 81: 96-104.

Gatley SJ, Pan D, Chen R, Chaturvedi G and Ding YS. Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. Life Sci 1996; 58: 231-9.

Gauggel S, Rieger M and Feghoff TA. Inhibition of ongoing responses in patients with Parkinson's disease. J Neurol Neurosurg Psychiatry 2004; 75: 539-44.

Gentsch C, Lichtsteiner M, Frischknecht HR, Feer H and Siegfried B. Isolation-induced locomotor hyperactivity and hypoalgesia in rats are prevented by handling and reversed by resocialization. Physiol Behav 1988; 43: 13-6.

Gerasimov MR, Franceschi M, Volkow ND, Gifford A, Gatley SJ, Marsteller D, Molina PE and Dewey SL. Comparison between intraperitoneal and oral methylphenidate administration: A microdialysis and locomotor activity study. J Pharmacol Exp Ther 2000; 295: 51-7.

Geyer MA and Markou A. Animal Models of Psychiatric Disorders. In: Bloom, FE and Kupfer, DJ. Psychopharmacology: The Fourth Generation of Progress, New York: Raven Press, Ltd., 1995, p 787-798.

Gilbert JG, Newman AH, Gardner EL, Ashby CR, Jr., Heidbreder CA, Pak AC, Peng XQ and Xi ZX. Acute administration of SB-277011A, NGB 2904, or BP 897 inhibits cocaine cue-induced reinstatement of drug-seeking behavior in rats: role of dopamine D3 receptors. Synapse 2005; 57: 17-28.

Glaser PE, Thomas TC, Joyce BM, Castellanos FX and Gerhardt GA. Differential effects of amphetamine isomers on dopamine release in the rat striatum and nucleus accumbens core. Psychopharmacology (Berl) 2005; 178: 250-8.

Goldman LS, Genel M, Bezman RJ and Slanetz PJ. Diagnosis and treatment of attention-deficit/hyperactivity disorder in children and adolescents. Council on Scientific Affairs, American Medical Association. Jama 1998; 279: 1100-7.

Good M and Honey RC. Dissociable effects of selective lesions to hippocampal subsystems on exploratory behavior, contextual learning, and spatial learning. Behav.Neurosci. 1997; 111: 487-493.

Green L and Myerson J. A discounting framework for choice with delayed and probabilistic rewards. Psychol Bull 2004; 130: 769-92.

Green L, Myerson J, Holt DD, Slevin JR and Estle SJ. Discounting of delayed food rewards in pigeons and rats: is there a magnitude effect? J Exp Anal Behav 2004; 81: 39-50.

Green L, Myerson J, Lichtman D, Rosen S and Fry A. Temporal discounting in choice between delayed rewards: the role of age and income. Psychol Aging 1996; 11: 79-84.

Green L, Myerson J and Macaux EW. Temporal discounting when the choice is between two delayed rewards. J Exp Psychol Learn Mem Cogn 2005; 31: 1121-33.

Green L, Myerson J and Ostaszewski P. Amount of reward has opposite effects on the discounting of delayed and probabilistic outcomes. J Exp Psychol Learn Mem Cogn 1999; 25: 418-27.

Greydanus DE, Sloane MA and Rappley MD. Psychopharmacology of ADHD in adolescents. Adolesc Med 2002; 13: 599-624.

Hahn B, Shoaib M and Stolerman IP. Nicotine-induced enhancement of attention in the five-choice

serial reaction time task: the influence of task demands. Psychopharmacology (Berl) 2002; 162: 129-37.

Hall FS, Huang S, Fong GW, Pert A and Linnoila M. Effects of isolation-rearing on locomotion, anxiety and responses to ethanol in Fawn Hooded and Wistar rats. Psychopharmacology (Berl) 1998; 139: 203-9.

Hard E, Carlsson SG, Jern S, Larsson K, Lindh AS and Svensson L. Behavioral reactivity in spontaneously hypertensive rats. Physiol Behav 1985; 35: 487-92.

Harrison AA, Everitt BJ and Robbins TW. Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: interactions with dopaminergic mechanisms. Psychopharmacology (Berl) 1997; 133: 329-42.

Harrison AA, Everitt BJ and Robbins TW. Central serotonin depletion impairs both the acquisition and performance of a symmetrically reinforced go/no-go conditional visual discrimination. Behav Brain Res 1999; 100: 99-112.

Hartig PR, Hoyer D, Humphrey PP and Martin GR. Alignment of receptor nomenclature with the human genome: classification of 5-HT1B and 5-HT1D receptor subtypes. Trends Pharmacol Sci 1996; 17: 103-5.

Heidbreder CA, Andreoli M, Marcon C, Thanos PK, Ashby CR, Jr. and Gardner EL. Role of dopamine D3 receptors in the addictive properties of ethanol. Drugs Today (Barc) 2004; 40: 355-65.

Heidbreder CA, Gardner EL, Xi ZX, Thanos PK, Mugnaini M, Hagan JJ and Ashby CR, Jr. The role of central dopamine D3 receptors in drug addiction: a review of pharmacological evidence. Brain Res Brain Res Rev 2005; 49: 77-105.

Hellemans KG, Nobrega JN and Olmstead MC. Early environmental experience alters baseline and ethanol-induced cognitive impulsivity: relationship to forebrain 5-HT(1A) receptor binding. Behav Brain Res 2005; 159: 207-20.

Hendley ED. WKHA rats with genetic hyperactivity and hyperreactivity to stress: a review. Neurosci Biobehav Rev 2000; 24: 41-4.

Hendley ED, Wessel DJ, Atwater DG, Gellis J, Whitehorn D and Low WC. Age, sex and strain differences in activity and habituation in SHR and WKY rats. Physiol Behav 1985; 34: 379-83.

Ho MY, Mobini S, Chiang TJ, Bradshaw CM and Szabadi E. Theory and method in the quantitative analysis of "impulsive choice" behaviour: implications for psychopharmacology. Psychopharmacology (Berl) 1999; 146: 362-72.

Holt DD, Green L and Myerson J. Is discounting impulsive?. Evidence from temporal and probability discounting in gambling and non-gambling college students. Behav Processes 2003; 64: 355-367.

Isles AR, Humby T and Wilkinson LS. Measuring impulsivity in mice using a novel operant delayed reinforcement task: effects of behavioural manipulations and d-amphetamine. Psychopharmacology (Berl) 2003;

Jaroni JL, Wright SM, Lerman C and Epstein LH. Relationship between education and delay discounting in smokers. Addict Behav 2004; 29: 1171-5.

Johansen EB, Aase H, Meyer A and Sagvolden T. Attention-deficit/hyperactivity disorder (ADHD) behaviour explained by dysfunctioning reinforcement and extinction processes. Behav Brain Res 2002; 130: 37-45.

Johansen EB and Sagvolden T. Response disinhibition may be explained as an extinction deficit in an animal model of attention-deficit/hyperactivity disorder (ADHD). Behav Brain Res 2004; 149: 183-96.

Johansen EB and Sagvolden T. Slower extinction of responses maintained by intra-cranial self-stimulation (ICSS) in an animal model of attention-deficit/hyperactivity disorder (ADHD). Behav Brain Res 2005; 162: 22-31.

Johnson MW and Bickel WK. Within-subject comparison of real and hypothetical money rewards in delay discounting. J Exp Anal Behav 2002; 77: 129-46.

Jolly DC, Richards JB and Seiden LS. Serotonergic mediation of DRL 72s behavior: receptor subtype involvement in a behavioral screen for antidepressant drugs. Biol Psychiatry 1999; 45: 1151-62.

Jones GH, Marsden CA and Robbins TW. Increased sensitivity to amphetamine and reward-related stimuli following social isolation in rats: possible disruption of dopamine-dependent mechanisms of the nucleus accumbens. Psychopharmacology (Berl) 1990; 102: 364-72.

Kafka MP and Prentky RA. Attention-deficit/hyperactivity disorder in males with paraphilias and paraphilia-related disorders: a comorbidity study. J

Clin Psychiatry 1998; 59: 388-96; quiz 397.

Kalbag AS and Levin FR. Adult ADHD and substance abuse: diagnostic and treatment issues. Subst Use Misuse 2005; 40: 1955-81.

Kandel ER, Schwartz JH and Jessell TM. Principles of Neural Science. McGraw-Hill, 2000.

Kheramin S, Body S, Mobini S, Ho MY, Velazquez-Martinez DN, Bradshaw CM, Szabadi E, Deakin JF and Anderson IM. Effects of quinolinic acid-induced lesions of the orbital prefrontal cortex on inter-temporal choice: a quantitative analysis. Psychopharmacology (Berl) 2002; 165: 9-17.

Kieres AK, Hausknecht KA, Farrar AM, Acheson A, de Wit H and Richards JB. Effects of morphine and naltrexone on impulsive decision making in rats. Psychopharmacology (Berl) 2004; 173: 167-74.

Kirby KN, Petry NM and Bickel WK. Heroin addicts have higher discount rates for delayed rewards than non-drug-using controls. J Exp Psychol Gen 1999; 128: 78-87.

Knardahl S and Sagvolden T. Open-field behavior of spontaneously hypertensive rats. Behav Neural Biol 1979; 27: 187-200.

Knobelman DA, Kung HF and Lucki I. Regulation of extracellular concentrations of 5-hydroxytryptamine (5-HT) in mouse striatum by 5-HT(1A) and 5-HT(1B) receptors. J Pharmacol Exp Ther 2000; 292: 1111-7.

Konrad K, Gauggel S, Manz A and Scholl M. Inhibitory control in children with traumatic brain injury (TBI) and children with attention deficit/hyperactivity disorder (ADHD). Brain Inj 2000; 14: 859-75.

Krikorian R, Zimmerman ME and Fleck DE. Inhibitory control in Obsessive-Compulsive Disorder. Brain Cogn 2004; 54: 257-9.

Kuczenski R and Segal DS. Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. J Neurochem 1997; 68: 2032-7.

Kulikov A, Aguerre S, Berton O, Ramos A, Mormede P and Chaouloff F. Central serotonergic systems in the spontaneously hypertensive and Lewis rat strains that differ in the elevated plus-maze test of anxiety. J Pharmacol Exp Ther 1997; 281: 775-84.

Kuntsi J, Oosterlaan J and Stevenson J. Psychological mechanisms in hyperactivity: I. Response inhibition deficit, working memory impairment, delay aversion, or something else? J Child Psychol Psychiatry 2001; 42: 199-210.

Lapiz MD, Fulford A, Muchimapura S, Mason R, Parker T and Marsden CA. Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. Neurosci Behav Physiol 2003; 33: 13-29.

Ledgerwood L, Richardson R and Cranney J. D-cycloserine facilitates extinction of learned fear: effects on reacquisition and generalized extinction. Biol Psychiatry 2005; 57: 841-7.

Lee JH, Kim HT and Hyun DS. Possible association between serotonin transporter promoter region polymorphism and impulsivity in Koreans. Psychiatry Res 2003; 118: 19-24.

Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Keefe RS, Davis SM, Davis CE, Lebowitz BD, Severe J and Hsiao JK. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med 2005; 353: 1209-23.

Lijffijt M, Bekker EM, Quik EH, Bakker J, Kenemans JL and Verbaten MN. Differences between low and high trait impulsivity are not associated with differences in inhibitory motor control. J Atten Disord 2004; 8: 25-32.

Liu YP, Wilkinson LS and Robbins TW. Effects of acute and chronic buspirone on impulsive choice and efflux of 5-HT and dopamine in hippocampus, nucleus accumbens and prefrontal cortex. Psychopharmacology (Berl) 2004; 173: 175-85.

Logan GD. On the Ability to Inhibit Thought and Action: A User's Guide to the Stop Signal Paradigm. In: Dagenbach, D and Carr, TH. Inhibitory processes in attention, memory, and language, SanDiego: Academic Press, 1994, p.

Maidment ID. The use of antidepressants to treat attention deficit hyperactivity disorder in adults. J Psychopharmacol 2003; 17: 332-6.

Mason ST and Iversen SD. Effects of selective forebrain noradrenaline loss on behavioral inhibition in the rat. J Comp Physiol Psychol 1977; 91: 165-73.

Maughan B, Rowe R, Messer J, Goodman R and Meltzer H. Conduct disorder and oppositional defiant disorder in a national sample: developmental epidemiology. J Child Psychol Psychiatry 2004; 45: 609-21.

Mazur JE. Effects of rate of reinforcement and rate of change on choice behaviour in transition. Q J Exp Psychol B 1997; 50: 111-28.

Mitchell JM, Fields HL, D'Esposito M and Boettiger CA. Impulsive Responding in Alcoholics. Alcohol Clin Exp Res 2005; 29: 2158-2169.

Mitchell SH. Effects of short-term nicotine deprivation on decision-making: delay, uncertainty and effort discounting. Nicotine Tob Res 2004; 6: 819-28.

Mobini S, Body S, Ho MY, Bradshaw CM, Szabadi E, Deakin JF and Anderson IM. Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. Psychopharmacology (Berl) 2002; 160: 290-8.

Mobini S, Chiang TJ, Al-Ruwaitea AS, Ho MY, Bradshaw CM and Szabadi E. Effect of central 5-hydroxytryptamine depletion on inter-temporal choice: a quantitative analysis. Psychopharmacology (Berl) 2000a; 149: 313-8.

Mobini S, Chiang TJ, Ho MY, Bradshaw CM and Szabadi E. Effects of central 5-hydroxytryptamine depletion on sensitivity to delayed and probabilistic reinforcement. Psychopharmacology (Berl) 2000b; 152: 390-7.

Morikawa H, Manzoni OJ, Crabbe JC and Williams JT. Regulation of central synaptic transmission by 5-HT(1B) auto- and heteroreceptors. Mol Pharmacol 2000; 58: 1271-8.

Murphy P. Inhibitory control in adults with Attention-Deficit/Hyperactivity Disorder. J Atten Disord 2002; 6: 1-4.

Myers MM, Musty RE and Hendley ED. Attenuation of hyperactivity in the spontaneously hypertensive rat by amphetamine. Behav Neural Biol 1982; 34: 42-54.

Myerson J, Green L and Warusawitharana M. Area under the curve as a measure of discounting. J Exp Anal Behav 2001; 76: 235-43.

Nakamura K, Shirane M and Koshikawa N. Site-specific activation of dopamine and serotonin transmission by aniracetam in the mesocorticolimbic pathway of rats. Brain Res 2001; 897: 82-92.

NIBUD. [Geldzaken in de praktijk]. 2005.

Nigg JT, Willcutt EG, Doyle AE and Sonuga-Barke EJ. Causal heterogeneity in attention-deficit/hyperactivity disorder: do we need neuropsychologically impaired subtypes? Biol Psychiatry 2005; 57: 1224-30.

Noldus LP, Spink AJ and Tegelenbosch RA. EthoVision: a versatile video tracking system for automation of behavioral experiments. Behav Res Methods Instrum Comput 2001; 33: 398-414.

Noldus LP, Trienes RJ, Hendriksen AH, Jansen H and Jansen RG. The Observer Video-Pro: new software for the collection, management, and presentation of time-structured data from videotapes and digital media files. Behav Res Methods Instrum Comput 2000; 32: 197-206.

Oades RD. Dopamine may be 'hyper' with respect to noradrenaline metabolism, but 'hypo' with respect to serotonin metabolism in children with attention-deficit hyperactivity disorder. Behav Brain Res 2002; 130: 97-102.

Ohmura Y, Takahashi T and Kitamura N. Discounting delayed and probabilistic monetary gains and losses by smokers of cigarettes. Psychopharmacology (Berl) 2005; 182: 508-15.

Okamoto K. Spontaneous hypertension in rats. Int Rev Exp Pathol 1969; 7: 227-70.

Olivier B, Mos J, Raghoebar M, de Koning P and Mak M. Serenics. Prog Drug Res 1994; 42: 167-308.

Olivier B, Mos J, van Oorschot R and Hen R. Serotonin receptors and animal models of aggressive behavior. Pharmacopsychiatry 1995; 28 Suppl 2: 80-90.

Olivier B and van Oorschot R. 5-HT(1B) receptors and aggression: A review. Eur J Pharmacol 2005; 526: 207-217.

Oosterlaan J and Sergeant JA. Inhibition in ADHD, aggressive, and anxious children: a biologically based model of child psychopathology. J Abnorm Child Psychol 1996; 24: 19-36.

Ortner CN, MacDonald TK and Olmstead MC. Alcohol intoxication reduces impulsivity in the delay-discounting paradigm. Alcohol Alcohol 2003; 38: 151-6.

Overtoom CC, Kenemans JL, Verbaten MN, Kemner C, van der Molen MW, van Engeland H, Buitelaar JK and Koelega HS. Inhibition in children with attention-deficit/hyperactivity disorder: a psychophysiological study of the stop task. Biol Psychiatry 2002; 51: 668-76.

Overtoom CC, Verbaten MN, Kemner C, Kenemans JL, van Engeland H, Buitelaar JK, van der

Molen MW, van der Gugten J, Westenberg H, Maes RA and Koelega HS. Effects of methylphenidate, desipramine, and L-dopa on attention and inhibition in children with Attention Deficit Hyperactivity Disorder. Behav Brain Res 2003; 145: 7-15.

Ozonoff S and Strayer DL. Inhibitory function in nonretarded children with autism. J Autism Dev Disord 1997; 27: 59-77.

Pare WP. Stress ulcer and open-field behavior of spontaneously hypertensive, normotensive, and Wistar rats. Pavlov J Biol Sci 1989; 24: 54-7.

Paredes RG and Agmo A. Has dopamine a physiological role in the control of sexual behavior? A critical review of the evidence. Prog Neurobiol 2004; 73: 179-226.

Parsons LH, Weiss F and Koob GF. Serotonin1b receptor stimulation enhances dopamine-mediated reinforcement. Psychopharmacology (Berl) 1996; 128: 150-60.

Pataki CS, Feinberg DT and McGough JJ. New drugs for the treatment of attention-deficit/hyperactivity disorder. Expert Opin Emerg Drugs 2004; 9: 293-302.

Pattij T, Broersen LM, van der Linde J, Groenink L, van der Gugten J, Maes RA and Olivier B. Operant learning and differential-reinforcement-of-low-rate 36-s responding in 5-HT1A and 5-HT1B receptor knockout mice. Behav Brain Res 2003; 141: 137-45.

Pattij T, de Jong TR, Uitterdijk A, Waldinger MD, Veening JG, Cools AR, van der Graaf PH and Olivier B. Individual differences in male rat ejaculatory behaviour: searching for models to study ejaculation disorders. Eur J Neurosci 2005; 22: 724-34.

Pelham WE, Gnagy EM, Chronis AM, Burrows-MacLean L, Fabiano GA, Onyango AN, Meichenbaum DL, Williams A, Aronoff HR and Steiner RL. A comparison of morning-only and morning/late afternoon Adderall to morning-only, twice-daily, and three times-daily methylphenidate in children with attention-deficit/hyperactivity disorder. Pediatrics 1999; 104: 1300-11.

Perry JL, Larson EB, German JP, Madden GJ and Carroll ME. Impulsivity (delay discounting) as a predictor of acquisition of IV cocaine self-administration in female rats. Psychopharmacology (Berl) 2004;

Petry NM. Delay discounting of money and alcohol in actively using alcoholics, currently abstinent alcoholics, and controls. Psychopharmacology (Berl) 2001a; 154: 243-50.

Petry NM. Pathological gamblers, with and without substance use disorders, discount delayed rewards at high rates. J Abnorm Psychol 2001b; 110: 482-7.

Petry NM, Kirby KN and Kranzler HR. Effects of gender and family history of alcohol dependence on a behavioral task of impulsivity in healthy subjects. J Stud Alcohol 2002; 63: 83-90.

Pietras CJ, Cherek DR, Lane SD, Tcheremissine OV and Steinberg JL. Effects of methylphenidate on impulsive choice in adult humans. Psychopharmacology (Berl) 2003a;

Pietras CJ, Cherek DR, Lane SD, Tcheremissine OV and Steinberg JL. Effects of methylphenidate on impulsive choice in adult humans. Psychopharmacology (Berl) 2003b; 170: 390-8.

Pliszka SR, McCracken JT and Maas JW. Catecholamines in attention-deficit hyperactivity disorder: current perspectives. J Am Acad Child Adolesc Psychiatry 1996; 35: 264-72.

Port RL and Seybold KS. Manipulation of NMDA-receptor activity alters extinction of an instrumental response in rats. Physiol Behav 1998; 64: 391-3.

Pothos EN, Creese I and Hoebel BG. Restricted eating with weight loss selectively decreases extracellular dopamine in the nucleus accumbens and alters dopamine response to amphetamine, morphine, and food intake. J Neurosci 1995; 15: 6640-50.

Potter AS and Newhouse PA. Effects of acute nicotine administration on behavioral inhibition in adolescents with attention-deficit/hyperactivity disorder. Psychopharmacology (Berl) 2004;

Przegalinski E, Filip M, Papla I and Czepiel K. Effects of 5-HT1B receptor ligands microinjected into the accumbal shell or core on the cocaine-induced locomotor hyperactivity in rats. J Physiol Pharmacol 2002; 53: 383-94.

Quay HC. Inhibition and attention deficit hyperactivity disorder. J Abnorm Child Psychol 1997; 25: 7-13.

Rachlin H, Raineri A and Cross D. Subjective probability and delay. J Exp Anal Behav 1991; 55: 233-44.

Ramirez JM and Andreu JM. Aggression, and some related psychological constructs (anger, hostility,

and impulsivity) Some comments from a research project. Neurosci Biobehav Rev 2005;

Ratey JJ and Gordon A. The psychopharmacology of aggression: toward a new day. Psychopharmacol Bull 1993; 29: 65-73.

Rescorla RA. Spontaneous recovery varies inversely with the training-extinction interval. Learn Behav 2004; 32: 401-8.

Resnick RJ. Attention deficit hyperactivity disorder in teens and adults: They don't all outgrow it. J Clin Psychol 2005;

Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, Hodges L and Davis M. Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. Arch Gen Psychiatry 2004; 61: 1136-44.

Retz W, Retz-Junginger P, Supprian T, Thome J and Rosler M. Association of serotonin transporter promoter gene polymorphism with violence: relation with personality disorders, impulsivity, and childhood ADHD psychopathology. Behav Sci Law 2004; 22: 415-25.

Retz W, Rosler M, Supprian T, Retz-Junginger P and Thome J. Dopamine D3 receptor gene polymorphism and violent behavior: relation to impulsiveness and ADHD-related psychopathology. J Neural Transm 2003; 110: 561-72.

Reynolds B, de Wit H and Richards J. Delay of gratification and delay discounting in rats. Behav Processes 2002; 59: 157.

Reynolds B, Richards JB, Horn K and Karraker K. Delay discounting and probability discounting as related to cigarette smoking status in adults. Behav Processes 2004; 65: 35-42.

Richards JB, Sabol KE and Seiden LS. DRL interresponse-time distributions: quantification by peak deviation analysis. J Exp Anal Behav 1993; 60: 361-85.

Richardson R, Ledgerwood L and Cranney J. Facilitation of fear extinction by D-cycloserine: theoretical and clinical implications. Learn Mem 2004; 11: 510-6.

Ridray S, Griffon N, Mignon V, Souil E, Carboni S, Diaz J, Schwartz JC and Sokoloff P. Coexpression of dopamine D1 and D3 receptors in islands of Calleja and shell of nucleus accumbens of the rat: opposite and synergistic functional interactions. Eur J Neurosci 1998; 10: 1676-86.

Rieger M and Gauggel S. Inhibition of ongoing responses in patients with traumatic brain injury. Neuropsychologia 2002; 40: 76-85.

Rieger M, Gauggel S and Burmeister K. Inhibition of ongoing responses following frontal, nonfrontal, and basal ganglia lesions. Neuropsychology 2003; 17: 272-82.

Robbins TW. The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology (Berl) 2002; 163: 362-80.

Rocha BA, Scearce-Levie K, Lucas JJ, Hiroi N, Castanon N, Crabbe JC, Nestler EJ and Hen R. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature 1998; 393: 175-8.

Rogoz Z, Skuza G and Kllodzinska A. Anxiolytic- and antidepressant-like effects of 7-OH-DPAT, preferential dopamine D3 receptor agonist, in rats. Pol J Pharmacol 2004; 56: 519-26.

Rogstad KE. Sex, sun, sea, and STIs: sexually transmitted infections acquired on holiday. Bmj 2004; 329: 214-7.

Rubinsztein JS, Rogers RD, Riedel WJ, Mehta MA, Robbins TW and Sahakian BJ. Acute dietary tryptophan depletion impairs maintenance of "affective set" and delayed visual recognition in healthy volunteers. Psychopharmacology (Berl) 2001; 154: 319-26.

Russell V, Allie S and Wiggins T. Increased noradrenergic activity in prefrontal cortex slices of an animal model for attention-deficit hyperactivity disorder--the spontaneously hypertensive rat. Behav Brain Res 2000a; 117: 69-74.

Russell V, de Villiers A, Sagvolden T, Lamm M and Taljaard J. Differences between electrically-, ritalin- and D-amphetamine-stimulated release of [3H]dopamine from brain slices suggest impaired vesicular storage of dopamine in an animal model of Attention-Deficit Hyperactivity Disorder. Behav Brain Res 1998; 94: 163-71.

Russell VA, de Villiers AS, Sagvolden T, Lamm MC and Taljaard JJ. Methylphenidate affects striatal dopamine differently in an animal model for attention-deficit/hyperactivity disorder--the spontaneously hypertensive rat. Brain Res Bull 2000b; 53: 187-92.

Sabol KE, Richards JB, Layton K and Seiden LS. Amphetamine analogs have differential effects on DRL 36-s schedule performance. Psychopharma-

cology (Berl) 1995; 121: 57-65.

Sagvolden T. Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD). Neurosci Biobehav Rev 2000; 24: 31-9.

Sagvolden T, Aase H, Zeiner P and Berger D. Altered reinforcement mechanisms in attention-deficit/hyperactivity disorder. Behav Brain Res 1998; 94: 61-71.

Sagvolden T, Hendley ED and Knardahl S. Behavior of hypertensive and hyperactive rat strains: hyperactivity is not unitarily determined. Physiol Behav 1992a; 52: 49-57.

Sagvolden T, Johansen EB, Aase H and Russell VA. A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/impulsive and combined subtypes. Behav Brain Sci 2005; 28: 397-419.

Sagvolden T, Metzger MA, Schiorbeck HK, Rugland AL, Spinnangr I and Sagvolden G. The spontaneously hypertensive rat (SHR) as an animal model of childhood hyperactivity (ADHD): changed reactivity to reinforcers and to psychomotor stimulants. Behav Neural Biol 1992b; 58: 103-12.

Sagvolden T, Pettersen MB and Larsen MC. Spontaneously hypertensive rats (SHR) as a putative animal model of childhood hyperkinesis: SHR behavior compared to four other rat strains. Physiol Behav 1993; 54: 1047-55.

Sahakian BJ, Robbins TW, Morgan MJ and Iversen SD. The effects of psychomotor stimulants on stereotypy and locomotor activity in socially-deprived and control rats. Brain Res 1975; 84: 195-205.

Sakaue M, Somboonthum P, Nishihara B, Koyama Y, Hashimoto H, Baba A and Matsuda T. Postsynaptic 5-hydroxytryptamine(1A) receptor activation increases in vivo dopamine release in rat prefrontal cortex. Br J Pharmacol 2000; 129: 1028-34.

Salamone JD and Correa M. Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. Behav Brain Res 2002; 137: 3-25.

Schachar R, Mota VL, Logan GD, Tannock R and Klim P. Confirmation of an inhibitory control deficit in attention-deficit/hyperactivity disorder. J Abnorm Child Psychol 2000; 28: 227-35.

Scheres A, Oosterlaan J, Geurts H, Morein-Zamir S, Meiran N, Schut H, Vlasveld L and Sergeant JA. Executive functioning in boys with ADHD: primarily an inhibition deficit? Arch Clin Neuropsychol 2004; 19: 569-94.

Scheres A, Oosterlaan J, Swanson J, Morein-Zamir S, Meiran N, Schut H, Vlasveld L and Sergeant JA. The effect of methylphenidate on three forms of response inhibition in boys with AD/HD. J Abnorm Child Psychol 2003; 31: 105-20.

Schipper J, Tulp MT and Sijbesma H. Neurochemical profile of eltoprazine. Drug Metabol Drug Interact 1990; 8: 85-114.

Schwartz JC, Diaz J, Bordet R, Griffon N, Perachon S, Pilon C, Ridray S and Sokoloff P. Functional implications of multiple dopamine receptor subtypes: the D1/D3 receptor coexistence. Brain Res Brain Res Rev 1998; 26: 236-42.

Seiden LS, Andresen J and MacPhail RC. Methylphenidate and d-amphetamine: effects and interactions with alphamethyltyrosine and tetrabenazine on DRL performance in rats. Pharmacol Biochem Behav 1979; 10: 577-84.

Sergeant JA, Geurts H and Oosterlaan J. How specific is a deficit of executive functioning for attention-deficit/hyperactivity disorder? Behav Brain Res 2002; 130: 3-28.

Silva FJ and Gross TF. The rich get richer: students' discounting of hypothetical delayed rewards and real effortful extra credit. Psychon Bull Rev 2004; 11: 1124-8.

Smith ER, Stefanick ML, Clark JT and Davidson JM. Hormones and sexual behavior in relationship to aging in male rats. Horm Behav 1992; 26: 110-35.

Sokoloff P, Andrieux M, Besancon R, Pilon C, Martres MP, Giros B and Schwartz JC. Pharmacology of human dopamine D3 receptor expressed in a mammalian cell line: comparison with D2 receptor. Eur J Pharmacol 1992a; 225: 331-7.

Sokoloff P, Giros B, Martres MP, Andrieux M, Besancon R, Pilon C, Bouthenet ML, Souil E and Schwartz JC. Localization and function of the D3 dopamine receptor. Arzneimittelforschung 1992b; 42: 224-30.

Solanto MV. Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration. Behav Brain Res 1998; 94: 127-52.

Solanto MV, Abikoff H, Sonuga-Barke E, Schachar R, Logan GD, Wigal T, Hechtman L, Hinshaw S

and Turkel E. The ecological validity of delay aversion and response inhibition as measures of impulsivity in AD/HD: a supplement to the NIMH multimodal treatment study of AD/HD. J Abnorm Child Psychol 2001; 29: 215-28.

Sonuga-Barke EJ. The dual pathway model of AD/HD: an elaboration of neuro-developmental characteristics. Neurosci Biobehav Rev 2003; 27: 593-604.

Sonuga-Barke EJ. Causal models of attention-deficit/hyperactivity disorder: from common simple deficits to multiple developmental pathways. Biol Psychiatry 2005; 57: 1231-8.

Sonuga-Barke EJ, Dalen L and Remington B. Do executive deficits and delay aversion make independent contributions to preschool attention-deficit/hyperactivity disorder symptoms? J Am Acad Child Adolesc Psychiatry 2003; 42: 1335-42.

Sonuga-Barke EJ, Taylor E, Sembi S and Smith J. Hyperactivity and delay aversion--I. The effect of delay on choice. J Child Psychol Psychiatry 1992; 33: 387-98.

Soubrié P. Reconciling the role of central serotonin neurones in human and animal behavior. Behav Brain Sci 1986; 319-64.

St Lezin E, Simonet L, Pravenec M and Kurtz TW. Hypertensive strains and normotensive 'control' strains. How closely are they related? Hypertension 1992; 19: 419-24.

Stewart JA and Tannock R. Inhibitory control differences following mild head injury. Brain Cogn 1999; 41: 411-6.

STIVORO. Smoking. The facts. [Roken. De harde feiten]. 2004; p 2

Tannock R, Schachar RJ, Carr RP, Chajczyk D and Logan GD. Effects of methylphenidate on inhibitory control in hyperactive children. J Abnorm Child Psychol 1989; 17: 473-91.

Thai DL, Yurasits LN, Rudolph GR and Perel JM. Comparative pharmacokinetics and tissue distribution of the d-enantiomers of para-substituted methylphenidate analogs. Drug Metab Dispos 1999; 27: 645-50.

Thanos PK, Katana JM, Ashby CR, Jr., Michaelides M, Gardner EL, Heidbreder CA and Volkow ND. The selective dopamine D3 receptor antagonist SB-277011-A attenuates ethanol consumption in ethanol preferring (P) and non-preferring (NP) rats. Pharmacol Biochem Behav 2005; 81: 190-7.

Thiebot MH, Le Bihan C, Soubrie P and Simon P. Benzodiazepines reduce the tolerance to reward delay in rats. Psychopharmacology (Berl) 1985; 86: 147-52.

Tobin H and Logue AW. Self-control across species (Columba livia, Homo sapiens, and Rattus norvegicus). J Comp Psychol 1994; 108: 126-33.

Trimbos Instituut. Een op de vijf verslaafden heeft ook ADHD; protocol ontwikkeld voor herkenning en behandeling [One in five addicts suffers from comorbid ADHD; protocol for diagnosis and treatment developed]. 2004; p

Tripp J and Viner R. Sexual health, contraception, and teenage pregnancy. Bmj 2005; 330: 590-3.

Troost PW, Lahuis BE, Steenhuis MP, Ketelaars CE, Buitelaar JK, van Engeland H, Scahill L, Minderaa RB and Hoekstra PJ. Long-term effects of risperidone in children with autism spectrum disorders: a placebo discontinuation study. J Am Acad Child Adolesc Psychiatry 2005; 44: 1137-44.

Turgay A. Treatment of comorbidity in conduct disorder with attention-deficit hyperactivity disorder (ADHD). Essent Psychopharmacol 2005; 6: 277-90.

van Boxtel GJ, van der Molen MW, Jennings JR and Brunia CH. A psychophysiological analysis of inhibitory motor control in the stop-signal paradigm. Biol Psychol 2001; 58: 229-62.

Van den Bergh FS, Bloemarts E, Chan JSW, Groenink L, Olivier B and Oosting R. Spontaneously Hypertensive Rats do not predict sumptoms of attention-deficit hyperactivity disorder. Pharmacol Biochem Behav 2005; Accepted for publication.

van Gaalen MM, van Koten R, Schoffelmeer AN and Vanderschuren LJ. Critical Involvement of Dopaminergic Neurotransmission in Impulsive Decision Making. Biol Psychiatry 2005;

Vergnes M, Depaulis A and Boehrer A. Parachlorophenylalanine-induced serotonin depletion increases offensive but not defensive aggression in male rats. Physiol Behav 1986; 36: 653-8.

Vergnes M, Depaulis A, Boehrer A and Kempf E. Selective increase of offensive behavior in the rat following intrahypothalamic 5,7-DHT-induced serotonin depletion. Behav Brain Res 1988; 29: 85-91.

Volkow ND, Wang G, Fowler JS, Logan J, Gerasimov M, Maynard L, Ding Y, Gatley SJ, Gifford

A and Franceschi D. Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. J Neurosci 2001; 21: RC121.

Vuchinich RE and Simpson CA. Hyperbolic temporal discounting in social drinkers and problem drinkers. Exp Clin Psychopharmacol 1998; 6: 292-305.

Wade TR, de Wit H and Richards JB. Effects of dopaminergic drugs on delayed reward as a measure of impulsive behavior in rats. Psychopharmacology (Berl) 2000; 150: 90-101.

Walderhaug E, Lunde H, Nordvik JE, Landro NI, Refsum H and Magnusson A. Lowering of serotonin by rapid tryptophan depletion increases impulsiveness in normal individuals. Psychopharmacology (Berl) 2002; 164: 385-91.

Walker DL, Ressler KJ, Lu KT and Davis M. Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. J Neurosci 2002; 22: 2343-51.

Weiss IC, Domeney AM, Heidbreder CA, Moreau JL and Feldon J. Early social isolation, but not maternal separation, affects behavioral sensitization to amphetamine in male and female adult rats. Pharmacol Biochem Behav 2001; 70: 397-409.

Wender EH. Attention-deficit/hyperactivity disorder: is it common? Is it overtreated? Arch Pediatr Adolesc Med 2002; 156: 209-10.

Wieland S, Boren JL, Consroe PF and Martin A. Stock differences in the susceptibility of rats to learned helplessness training. Life Sci 1986; 39: 937-44.

Wilens TE. AOD use and attention deficit/hyperactivity disorder. Alcohol Health Res World 1998; 22: 127-30.

Wilkinson LS, Killcross SS, Humby T, Hall FS, Geyer MA and Robbins TW. Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition. Neuropsychopharmacology 1994; 10: 61-72.

Will CC, Aird F and Redei EE. Selectively bred Wistar-Kyoto rats: an animal model of depression and hyper-responsiveness to antidepressants. Mol Psychiatry 2003; 8: 925-32.

Wilson JJ and Levin FR. Attention-deficit/hyperactivity disorder and early-onset substance use disorders. J Child Adolesc Psychopharmacol 2005; 15: 751-63.

Winstanley CA, Chudasama Y, Dalley JW, Theobald DE, Glennon JC and Robbins TW. Intraprefrontal 8-OH-DPAT and M100907 improve visuospatial attention and decrease impulsivity on the five-choice serial reaction time task in rats. Psychopharmacology (Berl) 2003a;

Winstanley CA, Dalley JW, Theobald DE and Robbins TW. Global 5-HT depletion attenuates the ability of amphetamine to decrease impulsive choice on a delay-discounting task in rats. Psychopharmacology (Berl) 2003b;

Winstanley CA, Dalley JW, Theobald DE and Robbins TW. Fractionating impulsivity: contrasting effects of central 5-HT depletion on different measures of impulsive behavior. Neuropsychopharmacology 2004a; 29: 1331-43.

Winstanley CA, Theobald DE, Cardinal RN and Robbins TW. Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. J Neurosci 2004b; 24: 4718-22.

Winstanley CA, Theobald DE, Dalley JW, Glennon JC and Robbins TW. 5-HT(2A) and 5-HT(2C) receptor antagonists have opposing effects on a measure of impulsivity: interactions with global 5-HT depletion. Psychopharmacology (Berl) 2004c;

Winstanley CA, Theobald DE, Dalley JW and Robbins TW. Interactions between serotonin and dopamine in the control of impulsive choice in rats: therapeutic implications for impulse control disorders. Neuropsychopharmacology 2005; 30: 669-82.

Wodushek TR and Neumann CS. Inhibitory capacity in adults with symptoms of Attention Deficit/Hyperactivity Disorder (ADHD). Arch Clin Neuropsychol 2003; 18: 317-30.

Wolff MC and Leander JD. Selective serotonin reuptake inhibitors decrease impulsive behavior as measured by an adjusting delay procedure in the pigeon. Neuropsychopharmacology 2002; 27: 421-9.

Wolraich ML, Hannah JN, Baumgaertel A and Feurer ID. Examination of DSM-IV criteria for attention deficit/hyperactivity disorder in a county-wide sample. J Dev Behav Pediatr 1998; 19: 162-8.

Wongwitdecha N and Marsden CA. Isolation rearing prevents the reinforcing properties of amphetamine in a conditioned place preference paradigm.

Eur J Pharmacol 1995; 279: 99-103.

Woolverton WL and Alling K. Choice under concurrent VI schedules: comparison of behavior maintained by cocaine or food. Psychopharmacology (Berl) 1999; 141: 47-56.

Wultz B, Sagvolden T, Moser EI and Moser MB. The spontaneously hypertensive rat as an animal model of attention-deficit hyperactivity disorder: effects of methylphenidate on exploratory behavior. Behav Neural Biol 1990; 53: 88-102.

Xi ZX, Gilbert J, Campos AC, Kline N, Ashby CR, Jr., Hagan JJ, Heidbreder CA and Gardner EL. Blockade of mesolimbic dopamine D3 receptors inhibits stress-induced reinstatement of cocaine-seeking in rats. Psychopharmacology (Berl) 2004; 176: 57-65.

Xi ZX, Gilbert JG, Pak AC, Ashby CR, Jr., Heidbreder CA and Gardner EL. Selective dopamine D3 receptor antagonism by SB-277011A attenuates cocaine reinforcement as assessed by progressive-ratio and variable-cost-variable-payoff fixed-ratio cocaine self-administration in rats. Eur J Neurosci 2005; 21: 3427-38.

Samenvatting in het Nederlands

Inleiding

Zonder impulsief gedrag, gedrag waar niet voldoende over nagedacht is, is het leven saai. Wanneer impulsiviteit in voldoende mate aanwezig is noemen we iemand ad rem of spontaan. Maar sommige mensen zijn continu impulsief, en het belet ze vrienden of hun baan te behouden. Zulk ernstig impulsief gedrag is een belangrijk kenmerk van veel psychiatrische stoornissen, zoals *attention-deficit hyperactivity disorder (ADHD)*, en *manische depressie*. Hoewel de *DSM-IV* impulsiviteit als één symptoom behandeld, zijn er aanwijzingen dat impulsiviteit een verzamelnaam is voor verschillende soorten gedrag. De verschillen tussen deze subtypen kunnen van groot belang zijn voor de behandeling van impulsiviteit.

De eerste soort impulsiviteit komt voort uit een onvermogen gedrag dat gepland was maar niet langer gewenst te inhiberen (remmen). Zo'n gebrek aan respons inhibitie is te meten met de zogenaamde stopsignaal *taak*. Een stopsignaal taak bestaat uit een groot aantal stimuluspresentaties (trials) waarin proefpersonen of proefdieren reageren op een stimulus. Ze drukken bijvoorbeeld op een knop wanneer er een lamp gaat branden. Dit noemen we een go-trial. In een aantal van die trials, de stop-trials, wordt er naast het visuele signaal een tweede signaal aangeboden, bijvoorbeeld een toon. Wanneer het subject deze toon hoort is het de bedoeling juist niet op de knop te drukken. Mensen kunnen deze taak na een aantal proeftrials prima uitvoeren, maar om dit gedrag een rat te leren zijn enkele maanden training nodig. De uitleesmaat van de stopsignaal taak is het succes waarmee geïnhibeerd wordt, maar ook kan de snelheid van het stopproces berekend worden. Om deze snelheid te berekenen gaan we er van uit dat een go- en een stopproces het tegen elkaar op nemen. De winnaar bepaalt het gedrag: wint het go-proces, dan wordt de respons uitgevoerd. Maar als het stopproces wint, dan lukt het om de respons in te houden. Omdat er bij een gebrek aan respons natuurlijk geen gedrag gemeten wordt, wordt de snelheid van het stopproces berekend op basis van de reactiesnelheid tijdens go-trials en het succes tijdens stop-trials. Met gebruik van de stopsignaal taak is in veel patiëntengroepen respons inhibitie gemeten. Het blijkt dat vooral bij ADHD-patiënten het stopproces vertraagd is, en dat *psychostimulantia* dit gedrag bovendien kunnen verbeteren.

Attention-deficit hyperactivity disorder (ADHD)
Stoornis gekenmerkt door aandachtsproblemen, hyperactiviteit en impulsiviteit. Hoewel de stoornis vooral voorkomt bij kinderen, kunnen de symptomen ook bij volwassenen voorkomen. Patiënten met ADHD worden vaak behandeld met methylfenidaat (zie beneden).

Manische depressie
Stoornis waarbij patiënten afwisselend perioden van depressie en manie doormaken. Impulsiviteit speelt vooral een rol tijdens de manische perioden.

DSM-IV
Handboek waarin de criteria staan beschreven voor de diagnose van alle psychische stoornissen.

Taak
Een gedragsmeting waarin de proefpersonen of proefdieren een opdracht uitvoeren wordt vaak een taak genoemd. Hun prestaties zijn de uitleesmaat. Mensen voeren taken vaak via de computer uit. Voor dieren wordt meestal gebruik gemaakt van een skinnerbox. Een skinnerbox is een experimentele opstelling waarin het gedrag van proefdieren (zoals het drukken op een pedaal) gemeten kan worden en diverse stimuli kunnen worden aangeboden (zoals voedsel, geluid of licht).

Psychostimulantia
Psychostimulantia vormen een klasse van farmaca die een stimulerende, activerende werking hebben op het gedrag. Amfetamine en methylfenidaat vallen allebei onder deze noemer. Paradoxaal verlagen psychostimulantia impulsiviteit in patiënten met ADHD, en vanaf de jaren 30 van de vorige eeuw worden psychostimulantia ook gebruikt om mensen af te remmen. Psychostimulantia werken vaak direct of indirect op het dopamine systeem (zie beneden)

Dopamine
Dopamine is een neurotransmitter: een boodschapperstof in het brein. Zenuwcellen communiceren door een neurotransmitter af te scheiden die vervolgens gebonden wordt door een receptor op de volgende zenuwcel. De meeste neurotransmitters binden aan meerdere receptoren. Dopamine bindt er aan vijf.

Mensen die lijden aan de tweede soort impulsiviteit geven de voorkeur aan directe beloningen, ook als wachten een aanzienlijk grotere beloning oplevert. Deze wachttijd wordt als onaangenaam ervaren, en impulsieve mensen zullen dus kiezen voor directe beloningen. Als dat niet mogelijk is, zullen ze hun aandacht afwenden van de wachttijd en hun doel, om zo de subjectieve wachttijd te verkorten. Het meten van dit soort impulsiviteit gebeurt in een uitgestelde-beloningen taak. In zo'n taak worden proefpersonen herhaaldelijk geconfronteerd met twee opties. Kiezen zij voor de eerste optie, dan krijgen zij direct een beloning (bijvoorbeeld 1 voerkorrel voor dieren, of een ingebeelde beloning van 60 euro voor mensen). Kiezen zij voor de tweede optie, dan krijgen zij een grotere beloning (bijvoorbeeld 4 voerkorrels, of een ingebeelde beloning van 150 euro), maar pas na een wachttijd (tot maximaal 1 minuut voor ratten, en 2 jaar voor mensen). Door de gemaakte keuzen te analyseren kan je de snelheid waarmee een beloning zijn waarde verliest als de beloning niet direct beschikbaar is, berekenen. Dit soort impulsiviteit komt ook voor bij patiënten die aan ADHD lijden, maar is vooral veel aangetoond in verslaafden. Het object van de verslaving blijkt niet zo veel uit te maken. Verslaafden aan nicotine, cocaïne, heroïne, alcohol, maar ook aan gokken, kiezen vaker voor directe, kleine beloningen dan uitgestelde, grotere beloningen.

Hoewel beide vormen van impulsiviteit waarschijnlijk onafhankelijk van elkaar zijn, verwachten we wel dat in de totstandkoming van beide vormen van impulsief gedrag dezelfde hersengebieden een rol spelen. Motivationele gebieden spelen vooral een rol in de uigestelde-beloningen taak. De hypothese is dat bij het kiezen voor een directe of een uitgestelde beloning de niet-gekozen optie geremd wordt. Dit is een vorm van respons inhibitie, en we verwachten dus dat de gebieden die een rol spelen bij respons inhibitie ook een rol spelen bij het kiezen voor de grote, uitgestelde of juist de kleine, directe beloning.

Hoofdstuk 1

In het eerste experimentele hoofdstuk wordt de *spontaan hypertensieve rat (SHR)* als *diermodel* voor ADHD onderzocht. In het begin van het onderzoeksproject waren de twee typen impulsiviteit nog niet zo duidelijk gedefiniëerd, en de methoden die gebruikt zijn om impulsiviteit te meten zijn dan ook niet heel erg selectief. Desondanks is wel duide-lijk dat deze methoden aspecten van impulsiviteit meten. In het *open veld* legden de SHR een grotere afstand af dan controledieren, maar deze hyperactiviteit verdween naarmate de dieren ouder werden. Bovendien werd deze hyperactiviteit niet verlaagd na toediening van *methylfenidaat*. In de DRL-72s, een taak voor impulsiviteit, krijgen de dieren een beloning wanneer zij op een pedaal drukken, maar alleen wanneer de laatste keer dat zij op dat pedaal drukten meer dan 72 seconden geleden was. Hierdoor worden de ratten gedwongen dit gedrag gedurende langere tijd te onderdrukken. Hoewel SHR minder beloningen kregen dan controledieren, en vaker snel achter elkaar meerdere responsen maakten, had methylfenidaat hier geen effect op. In hogere doseringen had methylfenidaat zelfs een negatief effect op het aantal behaalde beloningen. De vijf-keuzen taak is een taak voor aandacht en impulsiviteit, waarin dieren een wand met vijf lampjes in de gaten moeten houden. Zodra er een lampje aangaat, moet de rat zijn snuit in het bijbehorende gaatje steken. Doet hij dit goed, dan krijgt hij een beloning. Steekt hij zijn snuit in het verkeerde gat, dan wordt dit geïnterpreteerd als een aandachtsprobleem. Steekt hij zijn snuit in een gat nog voordat een lampje gaat branden, dan wordt dit geïnterpreteerd als impulsiviteit. SHR bleken in deze taak niet impulsief, en hebben ook geen aandachtstekort. Één van de controlegroepen bleek zelfs impulsiever in deze taak. Bovendien had methylfenidaat ook bij deze methode geen effect op het gedrag van de SHR. We concluderen dan ook dat de SHR geen goed model is, en we besloten geen gebruik meer te maken van dit soort modellen. Bovendien besloten we om specifiekere methoden te gebruiken om de twee typen impulsiviteit te meten.

Spontaan hypertensieve rat (SHR)

De spontaan hypertensieve rat is oorspronkelijk gefokt als modeldier (zie beneden) voor hoge bloeddruk. Deze dieren bleken echter ook zeer actief te zijn. Recent onderzoek laat zien dat de hoge bloeddruk en de hyperactiviteit die de SHR vertonen, onafhankelijk van elkaar veroorzaakt worden. Het is dan ook gelukt om SHR te fokken die geen hoge bloeddruk hebben.

Diermodel

Een model is een vereenvoudigde voorstelling van de werkelijkheid. Van een diermodel verwachten we dat zij één of meer symptomen vertonen die patiënten ook vertonen. Een andere eis is dat effectieve medicijnen bij de mens ook in deze dieren werken.

Open veld

Het open veld is een eenvoudige taak om activiteit te meten. De dieren worden in een bak gezet, en de afgelegde afstand wordt gemeten. Een groot voordeel aan de test is dat hij zo eenvoudig is uit te voeren. Een nadeel is dat het moeilijk te bepalen is wat je nu eigenlijk aan het meten bent.

Methylfenidaat

Methylfenidaat (Ritalin) is een stimulerende stof (zie Psychostimulantia) die voorgeschreven wordt aan kinderen en volwassenen met ADHD.

D-amfetamine
D-amfetamine valt ook onder de psychostimulantia (zie boven), en wordt soms gebruikt in patiënten die niet goed op methylfenidaat reageren. D-amfetamine wordt ook veel verkocht in het party-circuit, waar het vele namen kent, waaronder speed.

Fluvoxamine
Fluvoxamine is de eerste selectieve serotonine (zie beneden) heropname remmer (SSRI), een klasse van stoffen waaronder ook Prozac valt. SSRI's zijn vooral effectief bij depressie.

Serotonine
Serotonine is net als dopamine een neurotransmitter. Er bestaan 14 verschillende serotonine receptoren.

Maprotiline
Maprotiline is een noradrenaline heropname remmer. Naast psychostimulantia, is er in de Verenigde Staten een andere optie voor de behandeling van ADHD: atomoxetine. Maprotiline en atomoxetine hebben een vergelijkbaar mechanisme.

Noradrenaline
Noradrenaline is net als dopamine en serotonine een neurotransmitter.

Hoofdstuk 2

In hoofdstuk 2 beschrijven we de ontwikkeling van beide soorten impulsiviteit. Daarvoor hebben we twee methoden gebruikt. Ten eerste hebben we beide vormen van impulsiviteit gedurende een langere periode gemeten (ongeveer 10 maanden). Daarnaast hadden we de helft van de dieren alleen in een kooi gehuisvest, en de andere helft in groepen van vier (zoals gebruikelijk). Op deze manier werd de invloed van de sociale omgeving op impulsiviteit onderzocht. De uitleesmaten zijn de stopsignaal taak en de uitgestelde-beloning taak. De resultaten wezen uit dat respons inhibitie zeer stabiel was gedurende de gemeten periode. De voorkeur voor directe beloningen daalde gedurende de hele periode, maar onduidelijk blijft of de impulsiviteit echt afnam, of dat de dieren de taak op een andere manier gingen uitvoeren. Sociaal gehuisveste dieren bleken over betere respons inhibitie te beschikken dan geïsoleerd gehuisveste dieren. De voorkeur voor directe beloningen was aanvankelijk even groot in beide groepen dieren, maar na zes maanden onwikkelden de geïsoleerde dieren een grotere voorkeur voor directe beloningen dan groepsgehuisveste dieren.

In dit hoofdstuk wordt ook een begin gemaakt met de studie van de effecten van farmaca op beide vormen van impulsiviteit. Onder invloed van *D-amfetamine* kozen dieren vaker voor uitgestelde beloningen, maar deze zelfde stof verslechterde respons inhibitie. Het positieve effect van amfetamine was groter in de alleen gehuisveste dieren dan in de groepsgehuisveste dieren. *Fluvoxamine* had vrijwel geen effect op beide vormen van impulsiviteit. Ook *maprotiline* had geen effecten op beide vormen van impulsiviteit.

Hoofdstuk 3

In dit hoofdstuk worden twee belangrijke vragen onderzocht. Ten eerste vragen we ons af of de stopsignaal taak en de uitgestelde-beloning taak iets anders meten. Ten tweede vragen we ons af bij welk gedrag impulsiviteit, zoals gemeten met de twee genoemde taken, allemaal een rol speelt. Om deze twee vragen te beantwoorden hebben we een grote groep ratten beide taken geleerd zodat we de twee impulsiviteitstypen binnen hetzelfde dier kunnen meten. Daarna hebben we een aantal andere gedragingen gemeten die van belang zijn in een

rattenleven: *extinctieleren*, sexueel gedrag en aggressief gedrag.

Er was geen *correlatie* tussen respons inhibitie en voorkeur voor directe beloningen, wat aangeeft dat de twee concepten volkomen onafhankelijk zijn, en dat de twee taken verschillende zaken meten. Met andere woorden: weten hoe hoog een rat scoort op de stopsignaal taak geeft geen informatie over zijn voorkeur voor directe beloningen, en andersom. Extinctieleren bleek sterk gecorreleerd aan de voorkeur voor directe beloningen. Dit betekent dat dieren die veel responsen maakten tijdens extinctietrials, en daarvoor dus helemaal geen beloningen krijgen, ook vaak die dieren zijn die een voorkeur hebben voor de directe beloning boven de uitgestelde, maar veel grotere beloning. Sexueel gedrag was ongecorreleerd met beide typen impulsiviteit. Aggressie daarentegen, bleek sterk gecorreleerd aan een voorkeur voor de directe beloning. Dieren met een voorkeur voor de directe beloning bijten en vechten meer, en verwonden hun opponent meer.

Deze data geven inzicht in de betekenis van beide soorten impulsiviteit, maar zeker ook in de aard van extinctie en aggressie.

Hoofdstuk 4

In hoofdstuk 3 ontbreekt het verband tussen impulsiviteit en verslaving. Zoals beschreven in de inleiding, vermoeden we dat verslaving gekoppeld is aan een voorkeur voor directe beloningen. Daarom hebben we er voor gekozen dit verband te onderzoeken in mensen. We hebben met behulp van het internet 156 mensen ondervraagd over hun leeftijd, inkomen en verslavingen. Ook hebben we een vragenlijst afgenomen om hun voorkeur voor directe beloningen te meten. De proefpersonen werd daartoe gevraagd om steeds te kiezen tussen een kleine, direct beschikbare beloning (van 60, 90 of 120 euro), of een grote beloning van 150 euro, beschikbaar na een wachttijd (van een halve maand tot twee jaar).

Man of vrouw, oud of jong, heeft geen invloed. Of mensen zichzelf rijk of arm vinden daarentegen, bepaalde wel of ze een voorkeur hebben voor de directe of de uitgestelde beloning. Deze voorkeuren hadden geen invloed op alcoholconsumptie en gokgedrag. Rookgedrag en koffieconsumptie hingen wel samen met impulsiviteit. Rokers hadden een voorkeur voor directe beloningen vergeleken met niet-rokers. Koffiedrinkers

Extinctieleren

Wanneer gedrag niet langer belonend is zal het steeds minder vaak worden uitgevoerd. Dit uitdoven wordt extinctie genoemd. Bij extinctie wordt niets vergeten: in plaats daarvan wordt er iets nieuws geleerd. Er wordt geleerd dat een bepaald gedrag geen zin heeft. Extinctie is een basaal proces dat in ratten gemeten kan worden door ze eerste te trainen op een pedaal te drukken voor voer. Een dag later wordt het pedaal opnieuw aangeboden, maar responsen leveren geen beloning meer op.

Correlatie

Met een correlatie kan je het samen variëren van twee variabelen uitdrukken in een getal. Dit getal is een maat voor de variatie binnen een groep op een variabele, die verklaard kan worden uit een andere variabele, gemeten in diezelfde groep. Een positieve correlatie betekent dat een hoge score op de eerste variabele waarschijnlijk samen gaat met een hoge score op de tweede variabele. Een negatieve correlatie betekent dat een hoge score op de eerste variabele juist een voorspeller is voor een lage score op de tweede variabele.

Agonist
Communicatie tussen zenuwcellen in het brein vindt voor een belangrijk deel plaats doordat de ene cel een neurotransmitter vrijmaakt, en een volgende cel deze neurotransmitter bindt op een receptor. Een agonist is een stof die, wanneer hij bindt op een receptor in het brein, dezelfde werking uitlokt als wanneer een lichaamseigen neurotransmitter bindt op die receptor.

Eltoprazine
Eltoprazine is een anti-aggressieve stof. Deze klasse van stoffen worden wel de serenica genoemd. De werking van eltoprazine berust op activatie van bepaalde serotoninereceptoren.

Antagonist
Net als een agonist (zie boven) bindt een antagonist op receptoren. In tegenstelling tot een agonist doet een antagonist echter niets, maar door het bezetten van de receptor wordt de werking van de lichaamseigen neurotransmitter geblokkeerd.

leken impulsief wanneer zij een keuze maakten tussen 60 en 150 euro, maar leken minder impulsief waneer er gekozen moest worden tussen 120 en 150 euro. De conclusie is dat er een verband bestaat tussen sommige verslavingen en impulsiviteit. Waarom sommige verslavingen wel, en andere niet samengaan met een keuze voor de kleine, directe beloning, is onduidelijk. Wellicht speelt de intensiteit van de verslaving een rol. We verwachten, omdat onze steekproef vooral bestond uit hoogopgeleide mensen met een baan, dat alcoholverslaafden maar weinig voorkwamen in onze steekproef. Ook gokverslaafden zullen relatief zeldzaam zijn geweest. Rook- en koffieverslaafden daarentegen, komen veel voor, en functioneren vaak prima. De verwachting is dan ook dat zij veel voorkwamen in onze steekproef.

Hoofdstuk 5

Nu vastgesteld is dat impulsiviteit belangrijk is voor vele soorten gedragingen in hoofdstuk 3 en 4, en dat vooral het niet kunnen wachten op uitgestelde beloningen een voorname rol speelt in aggressie en verslaving, zitten we met de vraag of we hier wat aan kunenn doen. Daartoe hebben we zes potentieel werkzame stoffen getest in ratten die we de uitgestelde-beloning taak hebben geleerd. De zes geteste stoffen zijn D-amfetamine, 7-OH-DPAT (een dopamine D_3-*agonist*), flesinoxan (een *serotonine* 5-HT_{1A}-agonist), *eltoprazine* (een serotonine 5-HT1A/1B-agonist), GR127935 (een serotonine 5-HT_{1B}-*antagonist*), en D-cycloserine (een *NMDA-receptor* agonist). De keuze voor deze stoffen is gemaakt omdat de systemen waarop zij werken verband houden met motivatie (dopamine), aggressie (serotonine, vooral 5-$HT_{1A/1B}$), verslaving (dopamine D_3), en extinctie (NMDA).

Uit de resultaten blijkt dat D-amfetamine, zoals we eerder zagen in hoofdstuk 2, impulsiviteit verlaagd. De D_3-agonist 7-OH-DPAT heeft een impulsiviteitsverhogend effect, wat er op duidt dat een D_3-antagonist wellicht impulsiviteitsverlagende eigenschappen heeft. Flesinoxan ontregelde de prestaties van de dieren, waardoor zij vaker de kleine beloning kozen, ook als er helemaal geen wachttijd was. Eltoprazine, maar alleen bij een specifieke dosering, verlaagde impulsiviteit. Dit is geen verassing wanneer je bedenkt dat deze vorm van impulsiviteit gecorreleerd is aan aggressief gedrag (zie hoofdstuk 3). De

antagonist GR129935 had geen effect. D-cycloserine, een stof die effecten heeft op extinctie, had ook geen effect.

Hoofdstuk 6

Het gebruik van eltoprazine is mogelijk een effectieve remedie tegen impulsief gedrag, omdat ratten onder invloed van eltoprazine vaker voor de grote, maar uitgestelde beloning kiezen. In hoofdstuk 6 wordt wat dieper ingegaan op de effecten van eltoprazine. Eerst wordt gekeken naar de effecten van eltoprazine op beide vormen van impulsiviteit. Omdat deze effecten sterk lijken op die van D-amfetamine, is met behulp van *microdialyse* gekeken of dit effect misschien het gevolg is van veranderingen in dopaminerge neurotransmissie.

Respons inhibitie verslechterde na toediening van zowel eltoprazine als D-amfetamine. Dit bleek zowel uit een verlaging van het aantal correcte stop-trials als uit een vertraging van de stop reactietijd. Doseringen D-amfetamine hoger dan 1 mg/kg induceerden stereotype gedragingen, maar bij eltoprazine was dit niet het geval. Alle doseringen van eltoprazine leidden tot een toegenomen keuze voor de grote beloning. Bij D-amfetamine leek dit ook te gebeuren, maar bij sommige dieren induceerde D-amfetamine stereotypie, waardoor dit effect van D-amfetamine niet gemeten kon worden. De sterke overeenkomst tussen de effecten van eltoprazine en D-amfetamine doen vermoeden dat eenzelfde mechanisme wellicht aan beide effecten ten grondslag ligt. Uit de literatuur is bekend dat de effecten van D-amfetamine in uitgestelde-beloningen taken vooral te danken zijn aan de effecten van deze stof op het dopaminerge systeem. Met behulp van microdialyse werden geen veranderingen in het dopaminerge systeem gevonden, waardoor we konden uitsluiten dat de effecten van eltoprazine te wijten zijn aan dit systeem. Dit suggereert dat deze effecten direct aan serotonine te danken zijn. Door dit unieke mechanisme kan eltoprazine wellicht gebruikt worden om impulsiviteit te behandelen.

Discussie

Het onderzoeksproject zoals beschreven in dit proefschrift begint bij modellen en eindigt bij het potentieel werkzame eltoprazine. Twee hypothesen hebben aan dit project ten grondslag gelegen. De eerste hypothese is die van de opdeling

NMDA-receptor
Glutamaat is de meest voorkomende boodschapperstof in het brein, en er zijn een aantal receptoren waaraan glutamaat kan binden. Één zo'n receptor is de NMDA-receptor.

Microdialyse
Microdialyse is een veel gebruikte techniek waarbij een probe in het brein geplaatst wordt. Door deze probe stroomt een vloeistof, en het uiteinde van deze probe is uitgeriust met een semi-permeabel membraan. Boodschapperstoffen in het brein worden door de vloeistof opgenomen zodat later (relatieve) hoeveelheden bepaald kunnen worden.

van impulsiviteit in twee subtypen, en de manier waarop die subtypen samenhangen met elkaar en ander gedrag. De tweede gaat over de hersengebieden en neurotransmitters die betrokken zijn bij de subtypen van impulsiviteit.

De verkregen data ondersteunen de eerste hypothese. Zoals verwacht zijn de twee subtypen van impulsiviteit onafhankelijk van elkaar, en zijn ze afzonderlijk betrokken bij andere gedragingen. Het kunnen wachten op uitgestelde beloningen lijkt vooral belangrijk, en correleert met extinctie en aggressie. Zulke correlaties maken het mogelijk om in te schatten of een behandeling die gebruikt wordt voor een aandoening ook effectief gaat zijn bij een andere aandoening. De omvang van de correlatie kan zelfs dienen als maat voor het success dat je zult hebben wanneer je zo'n therapie toepast.

De tweede hypothese heeft ten grondslag gelegen aan veel onderzoek binnen dit project, maar de toetsing ervan is nog maar nauwelijks begonnen. In dit proefschrift komen een aantal hele duidelijk tekortkomingen van deze hypothese aan het licht. In het bijzonder werd het duidelijk dat het dopaminesysteem bij veel meer processen betrokken is dan verwacht, en dat de rol van serotonine veel ongrijpbaarder is. De hypothese is een nuttig kader waarin onderzoek geïnterpreteerd kan worden, maar vervolgonderzoek en aanpassing is nodig.

Thank you

All parts should go together without forcing.
You must remember that the parts you are reassembling
were disassembled by you. Therefore, if you can't get
them together again, there must be a reason.
By all means, do not use hammer.
IBM maintenance manual, 1975

Thank you Emilie, for doing such an excellent job with the animals and for being such a pleasant person to work with. Thank you Berend, for providing me with all the great opportunities and freedom that have led to this thesis. Thank you Leon, for your human touch and for always providing me with a new perspective. Thank you Ronald, for excellent supervision and introducing me to two great hobbies: the recumbent bicycle and art (even though I'm hopelessly untalented). Thank you Lucianne, for bringing me in, you have a wonderfully open mind. Thank you Jan, for introducing me to the Dom, I feel it's one of the things I do that really matters. Thank you Marja, for your efforts which laid the foundation for most of the chapters in this book, and for being such a good first student. Thank you Climmy, for your great skill with animals which lead to one of the more interesting chapters in this book. Thank you Audrey, for your work with those nasty mice who never want to do what we want them to do, I admire your dedication. Thank you Marjolein, for your excellent efforts on our highly experimental study, you are an excellent writer. Thank you Leila, for your enthusiasm, you are a quick learner and a hard worker. Thank you Bob, I enjoyed my stay in Denmark, and I hope we'll see each other again. Thank you Remco, without you this thesis would have been finished a year ago, but it wouldn't have been nearly as much fun. Thank you Johnny, for teaching me about the US, China, and for good advice on anything lab-animal related. Thank you Trynke, for the coffee. Thank you Koen W, for helping me on my biggest experiment, I greatly appreciated working with you, and I admire your diligence. Thank you Reinoud, for teaching me all I needed to know before starting my PhD, you taught me to think critically about what we are actually doing here. Thank you Dawn, for helping me set up the stop-signal task in our lab. Thank you Wim, for doing the testosterone determinations. Thanks to the rest of the department as well: Meg B, Liesbeth, Erik, Monika, Marijke, Anneloes, Mechiel, Anikò, you are all wonderful colleagues. Thank you Yair, for interesting conversations about many unlikely things, I'm looking forward to your defense and the lunch afterwards. Thank you Hans, for taking care of the animals, I'm glad I got to know you a bit better in the final six months. Thank you Koen B, I have often turned to you for the more interesting questions, and you always seem to have an answer as well. Thank you Marieke, our cooperation was really great, and I'm proud to look back and see that we actually did what we wanted to do four years ago. Thank you Anneke and Sven, you both provided invaluable support in the final stages of our projects. Thanks to everyone at the Instrumentele Dienst, you are swift, able, and always make me feel welcome. Thanks to everyone at the Dienst Grafische Vormgeving, for helping me out with my posters, and also this thesis. Thanks to everyone at Biostatistiek, I've followed nearly all courses for a reason, keep up the good work. Thank you Wiebe, for introducing me to Linux and maths. Thanks to everyone in the AIO opleidingscommissie and the departmentsraad, I've greatly enjoyed these diversions. Thank you Georgina, you contributed a nice translation to this book. Thank you Nap, unfortunately our project stranded, but I enjoy having you and Joyce as friends. Thank you Steven, for being a true and long-time friend, I greatly appreciate your solid, blunt friendliness. Thank you

Jeannie and Ton, for always being there. Thank you Ouke, you are a true friend, and I know I'll be more confident with you at my side at the defense and beyond. Thank you Simon, I hope I make you as proud as you make me, you are a great friend. And thank you Meg, for being there, for being you, and for countless other things I'd rather tell you in person over the next couple of decades than write down here for everybody to see.

About the author and co-authors

Co-authors and their affiliations (in order of appearance)

Drs. Filip S. van den Bergh
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University
Utrecht Institute for Pharmaceutical Sciences
Rudolf Magnus Institute of Neuroscience
(Curriculum vitae included at the end of this chapter)

Ing. Emilie M. Bloemarts
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University

Johnny S. W. Chan, BS
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University
Utrecht Institute for Pharmaceutical Sciences
Rudolf Magnus Institute of Neuroscience

Dr. Lucianne Groenink
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University
Utrecht Institute for Pharmaceutical Sciences
Rudolf Magnus Institute of Neuroscience

Prof. Dr. Berend Olivier
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University
Utrecht Institute for Pharmaceutical Sciences
Rudolf Magnus Institute of Neuroscience
Department of Psychiatry, Yale University School of Medicine, New Haven, U.S.A.
Psychogenics, Inc., Tarrytown, NY, U.S.A.

Dr. Ronald S. Oosting
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University
Utrecht Institute for Pharmaceutical Sciences
Rudolf Magnus Institute of Neuroscience

Climmy Cruijen
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University

Prof. Dr. J. Leon Kenemans
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University
Faculty of Social Science, Department of Psychonomics, Utrecht University
Utrecht Institute for Pharmaceutical Sciences
Helmholtz Institute

Marjolein Spronk
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University

Leila Ferreira, BS
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University

Drs. Marieke M. Lansbergen
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University
Faculty of Social Science, Department of Psychonomics, Utrecht University
Utrecht Institute for Pharmaceutical Sciences
Helmholtz Institute

Dr. Koen B. E. Böcker
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University
Utrecht Institute for Pharmaceutical Sciences
Helmholtz Institute

Ing. Koen G. C. Westphal
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University

Ing. André Klompmakers
Department of Psychiatry, University Medical Center Utrecht

Jasper Lipsch, MS
Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University

Prof. Dr. Herman G. M. Westenberg
Department of Psychiatry, University Medical Center Utrecht
Rudolf Magnus Institute of Neuroscience

Emer Leahy, PhD
Psychogenics, Inc., Tarrytown, NY, U.S.A.

About the author

Born on May 2, 1979 in Dordrecht, the Netherlands, I got my high school diploma at the Noordendijk in Dordrecht in 1997. In that same year, I started my study of Psychology at Utrecht University. I specialized in Biological Psychology, and during my internship at the Faculty of Pharmacy of the Universiteit Utrecht (now the Faculty of Sciences, department of Pharmaceutical Science), I studied conditioned fear in rats under the supervision of dr. Reinoud de Jongh and dr. Lucianne Groenink. During my studies, I worked as a teacher at the Faculty of Social Sciences twice: once as a statistics teacher, once as a methods teacher. After receiving my master's degree in early 2002, I joined dr. Koen Böcker at the Faculty of Pharmacy to study dark-enhanced startle in human subjects. In June 2002, I started my PhD project under supoervision of prof. dr. Berend Olivier, prof. dr. Leon Kenemans, dr. Ronald Oosting and dr. Lucianne Groenink at the Section Psychopharmacology of the Faculty of Sciences of Utrecht University, of which this thesis, exactly four years later, is the result.

During my employment at the Section Psychopharmacology, I participated in several meetings, including the Society for Neuroscience meeting and the Dutch Endo-Neuro-Psycho meeting. I organized a meeting on Impulsivity at Utrecht University. In addition, I founded and still maintain www.mednr.com, a free-for-all repository of computer programs used in animal conditioning. Finally, I am a member of both the PhD-educational committee and the departmental advisory body.

List of publications and selected abstracts

FS Van den Bergh, EM Bloemarts, JSW Chan, L Groenink, B Olivier, RS Oosting (2006). Spontaneously hypertensive rats do not predict symptoms of attention-deficit hyperactivity disorder. Pharmacology, Biochemistry and Behavior, in press.

FS Van den Bergh, C Cruijen, B Olivier, RS Oosting (2005). Effects of social isolation on two types of impulsivity. European Behavioural Pharmacology Society Abstract.

FS Van den Bergh, E Bloemarts, L Groenink, RS Oosting, B Olivier (2004). A behavioral and pharmacological assessment of the spontaneously hypertensive rat as a model for attention-deficit hyperactivity disorder. Society for Neuroscience Abstracts

FS Van den Bergh, L Groenink, J Van der Gugten, B Olivier (2003). Effects of methylphenidate on impulsivity of rats in the five choice serial reaction time task. Society for Neuroscience Abstracts.